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TITLE: Recombinant flagellin vaccines

Abstract Paragraph Left (1):
The present invention is directed to recombinant genes and their encoded proteins which are recombinant flagellin fusion proteins. Such fusion proteins comprise amino acid sequences specifying an epitope encoded by a flagellin structural gene and an epitope of a heterologous organism which is immunogenic upon introduction of the fusion protein into a vertebrate host. The recombinant genes and proteins of the present invention can be used in vaccine formulations, to provide protection against infection by the heterologous organism, or to provide protection against conditions or disorders caused by an antigen of the organism. In a specific embodiment, attenuated invasive bacteria expressing the recombinant flagellin genes of the invention can be used in live vaccine formulations. The invention is illustrated by way of examples in which epitopes of malaria circumsporozoite antigens, the B subunit of Cholera toxin, surface and presurface antigens of Hepatitis B, VP7 polypeptide of rotavirus, envelope glycoprotein of HIV, and M protein of Streptococcus, are expressed in recombinant flagellin fusion proteins which assemble into functional flagella, and which provoke an immune response directed against the heterologous epitope, in a vertebrate host.

Brief Summary Paragraph Right (23):
The present invention is directed to recombinant genes and their encoded proteins which are recombinant flagellin fusion proteins. Such proteins comprise an epitope encoded by a functional flagellin structural gene and at lease one epitope of a heterologous organism, which epitope is immunogenic upon introduction of the fusion protein into a vertebrate host. These epitopes are recognized by B cell and/or T cell epitopes. The epitope of a heterologous organism can be inserted into a region which is non-essential to function of the encoded flagellin, yet does not destroy its function, such as the hypervariable region of the flagellin structural gene. In a particularly preferred embodiment, the epitope of a heterologous organism is inserted between the natural EcoRV sites of the Salmonella H1-d gene. The recombinant flagellin proteins of the invention are exported to the cell surface, where, in a preferred embodiment, they assemble into functional flagella containing the heterologous epitope. In other embodiments, the recombinant flagellin fusion proteins of the invention can provoke a cellular, a mucosal, or a humoral response.

Brief Summary Paragraph Right (24):
The recombinant flagellin genes and proteins can be formulated for use as vaccines for protection against infection by the heterologous organism. They can also provide protection against conditions or disorders caused by an antigen of the heterologous organism. Expression as a recombinant flagellin fusion protein according to the present invention provides a method for presenting any desired epitope in an immunogenic form, to stimulate immune responses, including humoral, mucosal and/or cell-mediated immune responses. In a

specific embodiment, the recombinant flagellin genes of the invention can be expressed by attenuated invasive bacteria, in live oral vaccine formulations. In another specific embodiment, the recombinant flagellin fusion proteins can be formulated for use in subunit vaccines.

Brief Summary Paragraph Right (25):
In specific embodiments of the invention detailed in the examples section, epitopes of malaria circumsporozoite antigens, the B subunit of Cholera toxin, surface and presurface antigens of Hepatitis B, VP7 polypeptide of rotavirus, envelope glycoprotein of HIV, and M protein of Streptococcus are expressed on recombinant flagellin fusion proteins which assemble into functional flagella, and which provoke an immune response directed against the heterologous epitope, in a vertebrate host.

Drawing Description Paragraph Right (1):
FIG. 1. Diagrammatic representation of plasmids pLS402, pPX1651, and pLS408, encoding the H1-d flagellin structural gene. Plasmid pLS402 was isolated from a genomic library of Salmonella muenchen DNA constructed in pBR322 (Wei, L.-N. and Joys, T. M., 1985, J. Mol. Biol. 186:791). The coding region for the H1-d flagellin gene (darkened area) is present in a 3.8 kb EcoRI genomic fragment, and contains two EcoRV restriction sites. An additional EcoRV site is present on the vector. The two subclones, plasmids pPX1651 and pLS408, were constructed by first inserting the 3.8 kb genomic fragment of pLS402 into the EcoRI site of pUC18 and pUC19, respectively, resulting in constructions pPX1650 and pLS405, respectively. The 51 bp EcoRV fragment was then deleted from each of these plasmids, resulting in plasmids pPX1651 and pLS408, each of which now had a unique EcoRV restriction site available for insertion of oligonucleotides specifying a foreign epitope.

Drawing Description Paragraph Right (8):
FIG. 5. Schematic representation of the recombinant flagellin fusion proteins, constructed as described in Example 1. Cross-hatched areas represent the heterologous sequences, from the CS proteins of P. falciparum or P. berghei, or the B subunit or Cholera toxin (CT-B), as indicated.

Drawing Description Paragraph Right (9):
FIG. 6A-6D. Western Blot analysis of recombinant flagellins expressed in attenuated Salmonella. Cell extracts were electrophoresed and transferred to nitrocellulose filters as described in Example 1. The antibody probes used to detect recombinant flagellin molecules were: FIG. 6A: rabbit anti-H1-d antiserum; FIG. 6B: anti-P. berghei circumsporozoite Mab 3.28; FIG. 6C: anti-P. falciparum circumsporozoite Mab 4D9; FIG. 6D: rabbit anti-Cholera toxin amino acid residues 50-64 (CTP3 peptide) peptide serum. Plasmid constructions and host strains are indicated above each lane.

Drawing Description Paragraph Right (10):
FIG. 7. Detection of antibody to malaria circumsporozoite (CS) epitope in mice immunized with recombinant flagellin proteins. Mice were immunized and boosted with partially purified wild-type H1-d flagella (encoded by plasmid pPX1650) or recombinant flagella containing two copies of the P. berghei CS immunodominant repeat (encoded by plasmid pPX1661). Serial dilutions of sera obtained from these animals at weeks 0, 4 and 6 post primary immunization were assayed by ELISA for binding to synthetic peptides consisting of two copies of the P. berghei CS repeat coupled to keyhole limpet hemocyanin (KLH). Data presented are mean values calculated from five individual animals per group. a: plasmid pPXI650, at week 0; b: plasmid pPX1650 at week 4; c: plasmid pPX1650 at week 6; d: plasmid pPX1661 at week 0; e: plasmid pPX1661 at week 4; f: plasmid pPX1661 at week 6.

Drawing Description Paragraph Right (11):
FIG. 8. Detection of antibody to malaria circumsporozoite (CS) epitope in mice immunized with live attenuated Salmonella expressing recombinant flagellin fusion proteins. Mice were immunized and boosted as described in

Example 1. Serial dilutions of sera obtained from these animals at weeks 0, 4 and 6 post primary immunization were assayed by ELISA for binding to synthetic peptides consisting of two copies of the P. berghei CS repeat coupled to KLH. Data presented are mean values calculated from five individual animals per group, except for week 6, where only one animal remained per group. a: plasmid pPX1650, at week 0; b: plasmid pPX1650 at week 4; c: plasmid pPX1650 at week 6; d: plasmid pPX1662 at week 0; e: plasmid pPX1662 at week 4; f: plasmid pPX1662 at week 6.

Drawing Description Paragraph Right (17):
FIG. 14 shows data generated when SJL mice were primed with recombinant flagella, wild type flagella or CRM197 protein, and lymph node cells were restimulated in vitro with purified synthetic peptide encoding amino acids 366-383 of the CRM197 protein.

Detailed Description Paragraph Right (1):
The present invention relates to recombinant flagellin structural genes which are expressed as recombinant flagellin fusion proteins. Such recombinant genes comprise a sequence encoding an epitope specified by a flagellin structural gene and a sequence encoding an epitope of a heterologous organism, which epitope is immunogenic upon introduction of the fusion protein into a vertebrate host. The epitope of a heterologous organism can be inserted into a region which is non-essential to function of the encoded flagellin. However, such insert should not destroy flagellar function. In a preferred embodiment, the epitope of a heterologous organism can be inserted into the hypervariable region of the flagellin structural gene (e.g., between the natural EcoRV sites of the Salmonella H1-d gene).

Detailed Description Paragraph Right (2):
The invention also relates to the fusion flagellin proteins encoded by such genes, and the uses of these genes and proteins in vaccine formulations, for protection against infection by the heterologous organism or for protection against conditions or disorders caused by an antigen of the organism. Expression as a recombinant flagellin fusion protein according to the present invention provides a method for presenting any desired epitope in an immunogenic form, to stimulate immune responses (including humoral, mucosal and/or cell-mediated immune responses). In a specific embodiment, the recombinant flagellin genes of the invention can be expressed by attenuated invasive bacteria, in a live vaccine formulation. In another specific embodiment, the recombinant flagellin fusion proteins can be formulated for use in subunit vaccines.

Detailed Description Paragraph Right (3):
In specific embodiments of the invention detailed in the examples sections below, epitopes of malaria circumsporozoite antigens, the B subunit of Cholera toxin, surface and presurface antigens of Hepatitis B, VP7 polypeptide antigens of rotavirus, envelope glycoprotein of HIV, and M protein of Streptococcus pyogenes, are expressed on recombinant flagellin fusion proteins which assemble into functional flagella, and which provoke an immune response directed against the heterologous epitope, in a vertebrate host.

Detailed Description Paragraph Right (5):
The invention further pertains to a method of eliciting an immune response (including humoral, mucosal and/or cell-mediated immune responses) by administering to a vertebrate host, a bacterium transfected to express a recombinant flagellin fusion protein of this invention in a physiologically acceptable carrier. Preferably, the bacterium is live and infectious but cannot cause significant disease in the vertebrate host. Alternatively, the recombinant flagellar fusion protein itself can be administered to the host to elicit an immune response.

Detailed Description Paragraph Right (7):
Any flagellin structural gene can be used for the construction of a recombinant gene encoding a fusion flagellin protein containing a heterologous epitope. Such flagellin genes include but are not limited to the H1 and H2 genes of Salmonella, H of Bacillus subtilis and Pseudomonas aeruginosa, and hag of E. coli.

Detailed Description Paragraph Right (11):
Any DNA sequence which encodes an epitope of a heterologous organism, which when expressed as a flagellin fusion protein, produces protective immunity against such organism or against a condition or disorder caused by an

Detailed Description Paragraph Right (15):
In a preferred embodiment, the heterologous sequence encodes an immunopotent dominant epitope of a pathogen. In addition, molecules which are haptens (i.e., antigenic, but not immunogenic) may also be expressed as recombinant flagellin, since the flagellin may function as a carrier molecule in conferring immunogenicity on the hapten. Recombinant flagellins containing epitopes which are reactive with antibody although incapable of eliciting immune responses, still have potential uses in immunoassays.

Detailed Description Paragraph Right (16):
Peptides or proteins which are known to contain antigenic determinants can be incorporated into recombinant flagellins. If specific antigens are unknown, identification and characterization of immunoreactive sequences should be carried out. One way in which to accomplish this is through the use of monoclonal antibodies generated to the surface or other molecules of a pathogen. The peptide sequences capable of being recognized by the antibodies are defined epitopes. Alternatively, small synthetic peptides conjugated to carrier molecules can be tested for generation of monoclonal antibodies that bind to the sites corresponding to the peptide, on the intact molecule (see, e.g., Wilson, I. A., et al., 1984, Cell 37:767). Other methods known in the art which may be employed for the identification and characterization of antigenic determinants are also within the scope of the invention.

Detailed Description Paragraph Right (17):
In a specific embodiment, any DNA sequence which encodes a Plasmodium epitope, which when expressed as a flagellin fusion protein, is immunogenic in a vertebrate host, can be isolated for use according to the present invention. The species of Plasmodium which can serve as DNA sources include but are not limited to the human malaria parasites P. falciparum, P. malariae, P. ovale, P. vivax, and the animal malaria parasites P. berghei, P. yoelii, P. knowlesi, and P. cynomolgi. The antigens or fragments thereof which can be expressed as flagellin fusion proteins are antigens which are expressed by the malaria parasite at any of the various stages in its life cycle, such as the sporozoite, exoerythrocytic (development in hepatic parenchymal cells), asexual erythrocytic, or sexual (e.g., gametes, zygotes, ookinetes) stages. In a particular embodiment, the heterologous epitope to be expressed is an epitope of the circumsporozoite (CS) protein of a species of Plasmodium (see Example 1). Analogous CS proteins have been identified on the surfaces of sporozoites of all species of Plasmodium tested. Circumsporozoite protein antigens expressed in attenuated Salmonella spp. can be used as live vaccines directed against sporozoites, the invasive form of malaria parasites transmitted by the female Anopheles mosquito. Any epitope of a region of the CS protein important in the induction of protective humoral or cell-mediated immune response can be used in the vaccine formulations of the present invention. (See, e.g., Dame, J. B., et al., 1984, Science 225:593; Arnot, D. D., et al., 1985, Science 230:815; Weber et al., 1987, Exp. Parasitol. 63:295; Enea, V., et al., 1984, Science 225:628; Enea, V., et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:7520; Godson, G. N. et al., 1983, Nature 305:29; and McCutchan, T. F., et al., 1985, Science 230:1381 which references are incorporated by reference herein). For example, in one embodiment, the peptide asn-ala-asn-pro, representing the P. falciparum CS immunodominant repeating epitope, can be expressed by the recombinant bacteria of the invention. In another embodiment, the peptide asp-pro-ala-pro-pro-asn-ala-asn, representing the P. berghei CS protein immunodominant repeating epitope, can be expressed.

Detailed Description Paragraph Right (18):
In another specific embodiment, the Th2R epitope (Good, M. F., et al., 1987, Science 235:1059) of the P.

falciparum CS protein can expressed as a recombinant flagellin protein in the vaccine formulations of the present invention.

Detailed Description Paragraph Right (19):
In yet another embodiment, the heterologous epitope to be expressed as a recombinant flagellin fusion protein comprises a peptide of the B subunit of Cholera toxin. Suitable peptides are described by Jacob et al. As described by Jacob et al., (1983, Proc. Natl. Acad. Sci. U.S.A. 80:7611) peptides corresponding to several regions of the B subunit of cholera toxin have been synthesized and coupled to an immunogenic carrier in an effort to define epitopes which induce neutralizing antibodies. When these conjugates were used to raise antibodies in rabbits, one of these, encoding amino acids 50-64 (peptide CTP3), was shown to induce antibodies which recognized the native toxin and neutralized the biochemical (adenylate cyclase activation) and biological (intestinal fluid secretion) effects of the intact holotoxin (Jacob, C. O. et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:7893).

Detailed Description Paragraph Right (20):
Other epitopes which can be expressed as flagellin fusion proteins include but are not limited to the following: epitopes of the G protein of respiratory syncytial virus (RSV) (Collins et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:7683); neutralizing epitopes on Poliovirus I VP1 (Emini, E., et al., 1983, Nature 304:699); neutralizing epitopes on envelope glycoproteins of HIV I (Putney, S. D., et al., 1986, Science 234:1392-1395); epitopes present on Hepatitis B surface antigen (Itoh, Y., et al., 1986, Nature 308:19; Neurath., A. R., et al., 1986, Vaccine 4:34); epitopes of Diphtheria toxin (Audibert, F., et al., 1981, Nature 289:543); streptococcus 24M epitope (Beachey, E. H., 1985, Adv. Exp. Med. Biol. 185:193); and epitopes on gonococcal pilin (Rothbard, J. B. and Schoolnik, G. K., 1985, Adv. Exp. Med. Biol. 185:247).

Detailed Description Paragraph Right (21):
The flagellin fusion proteins in the vaccine formulations of the invention can also comprise an epitope of a heterologous organism, which when the fusion protein is introduced into a vertebrate host, induces an immune response that protects against a condition or disorder caused by an antigen containing the epitope. For example, in this embodiment of the invention, flagellin fusion proteins which encode an epitope of snake venom, bee venom, a hormone, sperm (for contraception), an allergy-inducing antigen or any other antigen to which an immune response is desired, may be used. In one particular embodiment, an epitope of an antigen of fat cell membranes can be expressed as a recombinant flagellin protein for formulation of a vaccine to decrease fat content in animals used as food sources. In another embodiment, a tumor-specific antigen can be expressed as a recombinant flagellin fusion protein, for induction of a protective immune response against cancer. In yet another embodiment, an epitope of a bacterial enterotoxin may also be expressed as a flagellin fusion protein. The nucleotide and deduced amino acid sequences for several bacterial enterotoxins have been determined (Mekalanos, J. J., et al., 1983 Nature 306:551; Leong, J., et al., 1985, Infect. Immun. 48:73).

Detailed Description Paragraph Right (22):
In another embodiment of the invention, DNA sequences encoding large regions of proteins which contain several B cell epitopes (i.e., epitopes capable of inducing a humoral immune response) and T cell epitopes (i.e., epitopes capable of inducing a cell-mediated immune response) can be introduced into the flagellin gene for expression as flagellin fusion proteins. By providing natural T helper cell epitopes as well as antibody-inducing epitopes, one can thus prime recipients for boosting by contact with a pathogenic heterologous organism.

Detailed Description Paragraph Right (23):
The gene sequences encoding the heterologous epitope to be expressed as a recombinant flagellin according to the present invention, can be isolated by techniques known in the art including but not limited to purification from genomic DNA of the microorganism, by cDNA synthesis from RNA of the microorganism, by recombinant DNA methods (Maniatis, T, et al., 1982, Molecular Cloning, A Laboratory Manual, Cold Spring

Harbor Laboratory, Cold Spring Harbor, N.Y.), or by chemical synthesis.

Detailed Description Paragraph Right (24):
In the construction of a recombinant flagellin gene of the present invention, sequences of a flagellin gene have sequences inserted into them or are replaced by a sequence(s) encoding an epitope(s) of a heterologous organism.

Detailed Description Paragraph Right (25):
First, the domains of the flagellin gene which are desired to have sequences inserted into them or are replaced by the heterologous sequences should be identified. Those flagellin sequences which are necessary and sufficient for transport of the flagellin protein to the distal end of the flagellum (or of the hook for initiation of a new flagellar filament) are desired to be conserved. This conservation results in a recombinant flagellin molecule which retains the ability to be expressed on the surface of the cell, as flagellar filament, thus facilitating isolation and purification of these recombinant molecules for use as components of a subunit vaccine, or facilitating their presentation to the immune system, in a live vaccine embodiment.

Detailed Description Paragraph Right (28):
In a preferred embodiment, a DNA sequence encoding a heterologous epitope is inserted into, or replaces, the central hypervariable region of the flagellin monomer. This embodiment allows the construction of recombinant flagellin monomers which may retain the ability to form intact flagella. The ability to assemble into flagella would, in the context of a live vaccine formulation, result in the presentation of a high concentration of the heterologous epitope, which exists on each flagellin monomer, to the immune system of the in vivo host. Presentation as an organized polymeric structure would afford a much stronger antigenic stimulus than the same material as monomer. Also, upon expression by a bacterium, presence of flagella on the external surface of the bacteria would allow a more effective presentation of the heterologous epitope. Furthermore, assembly into intact flagella would facilitate purification of the recombinant flagellin molecules, since various procedures for such purification are known in the art and may be used. In a most preferred embodiment, the recombinant flagellin molecules expressed by a parental nonmotile strain of bacteria produce functional flagella yielding motile bacteria which may thus more effectively present the heterologous epitope to the host immune system than a nonmotile strain, by virtue of the foreign epitope's presence on the external surface of the bacteria, and possibly the relatively greater invasiveness afforded by their motility.

Detailed Description Paragraph Right (31):
Many strategies known in the art can be used in the construction of the recombinant flagellin gene. For example, the relevant sequences of the flagellin gene and of the heterologous gene can, by techniques known in the art, be cleaved at appropriate sites with restriction endonuclease(s), isolated, and ligated. If cohesive termini are generated by restriction endonuclease digestion, no further modification of DNA before ligation may be needed. If however, cohesive termini of the DNA are not available for generation by restriction endonuclease digestion, or different sites other than those available are preferred, any of numerous techniques known in the art may be used to accomplish ligation of the heterologous DNA at the desired sites. For example, cleavage with a restriction enzyme can be followed by modification to create blunt ends by digesting back or filling in single-stranded DNA termini before ligation. Alternatively, the cleaved ends of the flagellin gene or heterologous DNA can be "chewed back" using a nuclease such as nuclease Bal 31, exonuclease III, lambda exonuclease, mung bean nuclease, or T4 DNA polymerase exonuclease activity, to name but a few, in order to remove portions of the sequence. An oligonucleotide sequence (a linker) which encodes one or more restriction sites can be inserted in a region of the flagellin gene by ligation to DNA termini. The subsequent ligation of a heterologous gene sequence into the cloning restriction site, so that both sequences are in the correct translational reading frame uninterrupted by translational stop signals, will result in a construct that directs the production of a flagellin fusion protein. A linker may also be used to generate suitable restriction sites in the heterologous gene sequence. Additionally, flagellin or heterologous gene sequences can be mutated

in vitro or in vivo in order to form new restriction endonuclease sites or destroy pre-existing ones, to facilitate in vitro ligation procedures. Any technique for mutagenesis known in the art can be used, including but not limited to, in vitro site-directed mutagenesis (Hutchinson, C., et al., 1978, J. Biol. Chem. 253:6551), use of TAB.TM. linkers (Pharmacia), etc.

Detailed Description Paragraph Right (32):
The particular strategy for constructing gene fusions will depend on the specific flagellin sequence to be replaced or inserted into, as well as the heterologous gene to be inserted.

Detailed Description Paragraph Right (33):
The recombinant flagellin gene should be constructed in or transferred into a vector which is capable of replication and expression in a bacterial host. In a preferred embodiment, the recombinant flagellin gene may also be inserted into the bacterial chromosomal DNA. One way in which this may be accomplished is by recombinational exchange with a plasmid-borne recombinant flagellin gene. In an alternative embodiment, the recombinant flagellin gene can be inserted into a cloning vector which can exist episomally, e.g., a plasmid or bacteriophage, which is then used to transform or infect appropriate host bacterial cells, where the recombinant DNA is replicated and expressed.

Detailed Description Paragraph Right (34):
The transformation of bacterial hosts with the DNA molecules that incorporate the recombinant flagellin gene enables generation of multiple copies of the flagellin sequence. A variety of vector systems may be utilized for expression within the bacterial host, including but not limited to plasmids such as pUC plasmids and derivatives, pBR322 plasmid and derivatives, bacteriophage such as lambda and its derivatives, and cosmids. In a specific embodiment, plasmid cloning vectors which can be used include derivatives of ColE1 type replicons (for additional information, see Oka et al., 1979, Mol. Gen. Genet. 172:151-159). The ColE1 plasmids are stably maintained in E. coli and Salmonella typhimurium strains as monomeric molecules with a copy number of about 15-20 copies per cell.

Detailed Description Paragraph Right (35):
Various regulatory expression elements can be used, which are any of a number of suitable transcription and translation elements that are active in bacteria. For instance, promoters which may be used to direct the expression of the recombinant flagellin sequence include but are not limited to the lactose operon promoter of E. coli, the hybrid trp-lac UV-5 promoter (tac) (DeBoer, H., et al., 1982, in Promoter Structure and Function, Rodriguez, R. L. and Chamberlain, M. J., eds., Praeger Publishing, New York), the leftward (P.sub.L) and the rightward (P.sub.R) promoters of bacteriophage lambda, the bacteriophage T7 promoter, the trp operon promoter, the lpp promoter (the E. coli lipoprotein gene promoter; Nakamura, K. and Inouye, I., 1979, Cell 18:1109-1117), etc. Other promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the inserted sequences. Alternatively, the native flagellin promoter may be used.

Detailed Description Paragraph Right (36):
Specific initiation signals are also required for efficient translation of inserted protein coding sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where the native flagellin gene sequences encoding its own initiation codon and adjacent sequences are inserted into the appropriate expression vectors, no additional translational control signals may be needed. However, in cases where the native flagellin translational signals are not present, exogenous translational control singals, including the ATG initiation codon, must be provided. The initiation codon must furthermore be in phase with the reading frame of the protein coding sequences to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic.

Detailed Description Paragraph Right (41):
The expression vector comprising the recombinant flagellin sequence should then be transferred into a bacterial host cell where it can replicate and be expressed. This can be accomplished by any of numberous methods known in the art including but not limited to transformation (e.g., of isolated plasmid DNA into the attenuated bacterial host), phage tranduction (Schmeiger, 1972, Mol. Gen. Genetics 119:75), conjugated between bacterial host species, electroporation, etc.

Detailed Description Paragraph Right (42):
In a specific embodiment, any attenuated bacterial hosts which express the recombinant flagellin can be formulated as live vaccines. Such bacteria include but are not limited to attenuated invasive strains and attenuated Campylobacter, Shigella or Escherichia species.

Detailed Description Paragraph Right (43):
In a preferred embodiment of the present invention, the expression vector comprising the recombinant flagellin sequence is transferred into an attenuated invasive bacteria, where it is expressed, thus producing a bacterial strain suitable for use as a live vaccine.

Detailed Description Paragraph Right (44):
Any of various attenuated invasive bacteria can be used as a vehicle to express the recombinant flagellin so that its heterologous epitope is effectively presented to the host immune system, in the vaccine formulations of the present invention. The vaccine bacteria retain their invasive properties, but lose in large part their virulence properties, thus allowing them to multiply in the host to a limited extent, but not enough to cause significant disease or disorder. Examples of invasive bacteria which, in attenuated forms, may be used in the vaccine formulations of the invention include but are not limited to Salmonella spp., invasive E. coli (EIEC), and Shigella spp. In a preferred embodiment, invasive bacteria which reside in lymphoid tissues such as the spleen (e.g., Salmonella spp.) are used. Such bacteria can invade gut epithelial tissue and/or Peyer's patches, disseminate throughout the reticuloendothelial system, and gain access to mesenteric lymphoid tissue, liver, and spleen, where they multiply or at least survive for a time, and induce humoral and cell-mediated immunity.

Detailed Description Paragraph Right (47):
In specific embodiments, Salmonella bacteria that have been attenuated by chromosomal deletion of gene(s) for aromatic compound biosynthesis (aro), or mutation in the galE gene, or that are cya.sup.-, crp.sup.- vir plasmid.sup.-, etc., can be used. Aro mutants which can be used include but are not limited to S. typhi strains 543Ty and 541Ty, for use in vaccines for humans, and S. typhimurium SL3261 and SL1479, and S. enteriditis serotype dublin SL1438, (also termed S. dublin) for use in animals. (See U.S. Pat. No. 4,550,081 for a description of S. typhimurium strain SL1479 and S. dublin strain SL1438). S. typhi strains such as 543Ty and 541Ty are avirulent in humans by virute of attenuation by deletion affecting genes aroA and/or purA (Levine, M. M., et al., 1987, J. Clin. Invest. 79:888). Mutants of S. dublin, such as SL1438, and of S. typhimurium, such as SL3261, can be used in the development of animal model systems, since these species are capable of causing animal diseases equivalent to typhoid fever. galE mutants which can be used include but are not limited to Salmonella typhi strains Ty21a (Germanier, 1984, Bacteria Vaccines, Academic Press, NY pp. 137-165) Salmonella typhimurium G30D, etc.

Detailed Description Paragraph Right (48):
In a preferred embodiment, a plasmid expression vector containing a recombinant flagellin gene can be isolated and characterized in E. coli, before transfer to an attenuated Salmonella stain, e.g., by phage transduction (Schmeiger, 1972, Mol. Gen. Genetics 119:75), because of the high transformation frequencies of E. coli K12 relative to those of Salmonella such as S. typhimurium.

Detailed Description Paragraph Right (49):

Immunopotency of the heterologous epitope expressed as a recombinant flagellin, in its live vaccine formulation, can be determined by monitoring the immune response of test animals following immunization with bacteria expressing the recombinant flagellin. In a subunit vaccine formulation, the immune response of test animals can be monitored following immunization with the isolated recombinant flagellin molecule, as flagellar filaments or monomer, which can be formulated with an appropriate adjuvant to enhance the immunological response. Suitable adjuvants include, but are not limited to, mineral gels, e, aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, and potentially useful human adjuvants such as BCG (Bacille Calmette-Guerin) and Corynebacterium parvum. Test animals may include mice, guinea pigs, rabbits, chickens, chimpanzees and other primates, and eventually human subjects. Methods of introduction of the immunogen may include oral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal or any other standard routes of immunizations.

Detailed Description Paragraph Right (51):
In this embodiment of the invention, the recombinant flagellins which comprise an epitope of a heterologous organism are formulated for vaccine use. Such vaccines formulations can comprise live vaccines or subunit vaccine formulations. The vaccine formulations of the invention are of use in both animals and humans.

Detailed Description Paragraph Right (52):
The purpose of this embodiment of the invention is to formulate a vaccine in which the immunogen is an attenuated invasive bacterial strain that expresses a recombinant flagellin comprising an epitope of a heterologous organism so as to elicit an immune (humoral and/or cell-mediated) response to the heterologous epitope that will protect against infections by the organism or conditions or disorders caused by an antigen of the organism. The bacteria of the vaccine comprise strains that are infectious for the host to be vaccinated. In a preferred embodiment, such strains are attenuated invasive bacteria such as Salmonella species. Other suitable species can include but are not limited to Shigella and E. coli. In a most preferred embodiment, the recombinant flagellin genes are expressed by the host bacteria as flagellin monomers that assemble into functional flagella, allowing the heterologous epitope on the recombinant molecules to be presented in a large number of copies to the host immune system.

Detailed Description Paragraph Right (54):
the same recombinant flagellin protein, on separate recombinant flagellin molecules encoded by the same or different expression vectors, or in different bacteria.

Detailed Description Paragraph Right (56):
In specific embodiments, attenuated Salmonella expressing a recombinant flagellin comprising an epitopes of a malarial circumsporozoite protein, the B subunit of cholera toxin, surface and presurface antigens of Hepatitis B, VP7 polypeptide of rotavirus, envelope glycoprotein of HIV, and M protein of Streptococcus can be formulated as vaccines.

Detailed Description Paragraph Right (58):
The heterologous peptide expressed as a recombinant flagellin fusion protein, may be used as an immunogen in subunit vaccine formulations, which may be multivalent. The multivalent vaccine formulation can comprise recombinant flagella, or a recombinant flagellin monomer containing more than one heterologous epitope, which epitope may be of different organisms, or several flagellin molecules, each encoding a different heterologous epitope, etc.

Detailed Description Paragraph Right (59):
The recombinant flagellin gene product may be purified for purposes of vaccine formulation from any vector/host systems that express the heterologous protein, such as transduced or transformed bacteria. For example, bacterial flagellar filaments are easily removed from the intact bacterium by mechanical means

which do not otherwise damage the cell, thus allowing them to be easily purified without introducing harsh, denaturing agents. Standard procedures known in the art can be used for the purification of recombinant flagellin, either as monomers or as (assembled) flagella (see e.g., Gill, P. R., and Agablan, N., 1983, J. Biol. Chem. 258:7395-7401; Weissborn, A., et al., 1982, J. Biol. Chem. 257:2066-2074; Gill, P. R., and Agabian, N., 1982, J. Bacteriol. 150:925-933; Lagenaur, C., and Agabian, N., 1976, J. Bacteriol. 128:435-444; Fukuda, A., et al., 1978, FEBS Lett. 95:70-75; Stevenson, J. R., and Stonger, K. A., 1980, Am. J. Vet. Res. 41(4):650-653).

Detailed Description Paragraph Right (60):
Furthermore, isolated flagella samples can be solubilized (e.g., by dissociation upon exposure either to pH 3 or to pH 11 at low ionic strength; DeLange, R. J., et al., 1976, J. Biol. Chem. 251(3):705-711) to flagellin subunits and then reassociated to flagella by known procedures (eg, Weissborn, A., et al., 1982, J. Biol. Chem. 257:2066-2074) in order to: (a) aid in the purification of the recombinant flagellins by removing undesirable contaminants; and/or (b) produce an immunogen for multivalent vaccine formulation, by association of recombinant flagellin monomers encoding different heterologous epitopes.

Detailed Description Paragraph Right (62):
In instances where the recombinant flagellin gene product is a hapten, i.e., a molecule that is antigenic in that it can react selectively with cognate antibodies, but not immunogenic in that it cannot elicit an immune response, the hapten may covalently bound to a carrier or immunogenic molecule; for instance, a large protein such as serum albumin will confer immunogenicity to the hapten coupled to it. The hapten-carrier may be formulated for use as a vaccine.

Detailed Description Paragraph Right (64):
The antibodies generated against heterologous organisms by immunization with the recombinant flagellin of the present invention also have potential uses in diagnostic immunoassays, passive immunotherapy, and generation of antiidiotypic antibodies.

Detailed Description Paragraph Right (68):
The recombinant flagellin gene products of the present invention, or fragment thereof, expressing foreign epitope(s), may be used as antigens in immunoassays for the detection of antibodies to the epitope(s). The heterologous protein, or fragments thereof, may also be used to detect the same or related epitope(s) by competition assays. The recombinant flagellin products, or the foreign epitope(s) expressed by them, may be used in any immunoassay system known in the art including but not limited to competitive and noncompetitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme-linked immunosorbent assay), "sandwich" immunoassay, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays and immunoelectrophoresis assays, to name but a few.

Detailed Description Paragraph Right (72):
Each motile strain was used as recipient in transduction, with SL5669, which is an S. typhimurium strain with transposon Tn10 inserted in gene H1-i, for its phase-1 flagellar antigen, i. Selection was made for clones which were resistant to tetracycline, because of replacement of gene H1-g,p of the recipient by gene H1-i::Tn10 of the donor. A tetracycline resistant clone, found nonmotite (because of replacement of the wild-type flagellin gene by the gene inactivated by the transposon) and free of the phage, P22 HT105/1 used to effect the transduction, was retained, SL5927 from the cross with SL1438 as recipient and SL5928 from that with SL5631 as recipient.

Detailed Description Paragraph Right (73):
SL5631 is a stable aromatic-dependent derivative of a virulent S. dublin strain, SVA47. It was obtained by two steps of transduction, by the method used to construct aroA (deletion) strains of S. typhi (Edwards, M.

F., 1985, Ph.D. Thesis, Stanford University, California).

Detailed Description Paragraph Right (74):
The construction and expression of recombinant flagellin genes encoding foreign epitopes important in the induction and expression of protective immune responses is described. The heterologous parasitic and bacterial epitopes which were expressed as recombinant flagellin were of the malarial CS protein, and of the B subunit of cholera toxin. The recombinant flagellin molecules were introduced into and expressed by attenuated Salmonella strains, which can be used in live vaccine formulations.

Detailed Description Paragraph Right (75):
The bacterial strains used were Salmonella strains SL1438 (ATCC Accession No. 39184) and SL5927 (ATCC Accession No. 67944), and E. coli strain CL447. Plasmid pLS402 contains a 3.8 kb EcoRI fragment of genomic DNA encoding the complete H1-d flagellin structural gene from S. muenchen inserted into the EcoRI site of plasmid pBR322 (Wei, L.-N. and Joys, T. M., 1985, J. Mol. Biol. 186:791). Plasmid pUC18, pUC19, and E. coli strain JM103 were obtained from Bethesda Research Laboratories (BRL; Bethesda, Md.).

Detailed Description Paragraph Right (91):
To analyze recombinant flagellin proteins by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), 500 microliters of an overnight culture of bacteria containing a recombinant plasmid were centrifuged, and the pellet was resuspended in 200 microliters of protein running mix (0.125 M Tris-HCl, pH 6.8, 2.5% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.005% bromophenol blue), and heated to 100.degree. C. for 10 minutes. 20 microliters of each samples were electrophoresed under conditions described by Laemmli (Laemmli, U. K., 1979, Nature 227:680), through a stacking gel of 4% acrylamide and a separating gel of 10% acrylamide.

Detailed Description Paragraph Right (94):
Overnight cultures of S. dublin SL1438 harboring recombinant plasmids were used to inoculate 150 mm petri dishes containing 1.5% (w/v) Difco agar in LB medium supplemented with 100 ug/ml ampicillin, and plates were incubated for 48 hours at 37.degree. C. These plates were then flooded with deionized water, and bacteria were gently removed from the surface by scraping. This suspension was blended at high speed in a standard food blender, and bacterial debris was removed by centriguation at 10,000 rpm in a Sorvall SS34 rotor for 30 minutes. Flagella present in the supernate were concentrated by ultracentrifugation at 50,000 rpm in a Beckman 70.1Ti rotor for one hour. These preparations of flagella were judged to be approximately 90% pure by Coomassie blue protein staining of SDS-PAGE gels, and protein concentrations were estimated by comparison with known amounts of standard proteins run on the same gels.

Detailed Description Paragraph Right (99):
Plasmid pLS402 contains a 3.8 kb EcoRI fragment of genomic DNA which includes the complete H1-d (H1 antigen d) flagellin structural gene (Wei, L.-N and Joys, T. M., 1985, J. Mol. Biol. 186:791-803) (FIGS. 2A, 2B) from S. muenchen (American Type Culture Collection Accession No. 8388) inserted into the EcoRI site of plasmid pBR322 (FIG. 1; Wei, L.-N., and Joys, T. M., 1985, J. Mol. Biol. 186:791). Examination of the published base sequence of the coding region for this gene (FIG. 2B) revealed two EcoRV restruction sites separated by 48 bp at positions 619 and 667. By comparison with sequences derived from other H1 genes, the region of the gene containing these two restriction sites was demonstrated to be highly variable in both primary amino acid sequence and in the number of residues. We thus concluded that this region of the gene may be dispensable for flagella assembly and function, and would thus be an appropriate location for the insertion of DNA encoding foreign epitopes. In order to utilize this strategy, it was necessary to subclone the H1-d gene onto a plasmid vehicle which did not have any EcoRV restriction sites. Therefore, the 3.8 kb EcoRI fragment of pLS402 was isolated and subcloned into the EcoRI site of pUC18 and of pUC19, resulting in the construction of plasmids pPX1650 and pLS405, respectively. These latter vectors could then be used to exchange the

authetic H1-d DNA between nucleotide numbers 619 and 667 (FIG. 2B) for synthetic or cloned DNA encoding a foreign epitope. In order to further facilitate the screening of recombinant plasmids, the 48 bp fragment between the EcoRV sites in each plasmid was deleted by digesting pPX1650 and pLS405 with EcoRV and religating each of the digested plasmids. Transformants were then screened for the loss of the 48 bp fragment; pPX1651 and pLS408 were thus obtained (FIG. 1). These plasmids retained only a single EcoRV site for insertion of foreign epitopes. In addition, it was now possible to distinguish, by size, a vector with an insertion of a 48 bp piece of foreign DNA from a vector which had simply religated to itself.

Detailed Description Paragraph Right (100):
To test the ability of foreign epitopes to be expressed as genetic fusions with flagellin, several genetic constructions were made, as described infra.

Detailed Description Paragraph Right (101):
Recombinant flagellin genes were constructed which encoded epitopes of malaria parasite (genus Plasmodium) circumsporozoite proteins as flagellin fusion proteins.

Detailed Description Paragraph Right (102):
Initially, two complementary 48-residue oligonucleotides were synthesized encoding four copies of the P. falciparum circumsporozoite protein four-amino-acid repeat sequence (FIG. 3A). The sequences of these nucleic acid fragments were such that, when annealed, complementary three base overhangs were created which allowed oligonucleotides to ligate to themselves only in a head to tail fashion, thus ensuring insertion of multiple oligonucleotides all in the same orientation. After the fragments were annealed, they were blunt-ended by treatment with DNA polymerase large fragment (Klenow enzyme), and ligated to pPX1651 which had been digested previously with EcoRV. Following transformation of E. coli strain JM103, ampicillin-resistant colonies were screened for the presence of recombinant plasmids by restruction site analysis of plasmid DNA. Two recombinants were identified: pPX1653 contains a single copy of the inserted oligonucleotide, and pPX1652 contains three inserted oligonucleotides. By DNA sequence analysis, the three oligonucleotide fragments present in pPX1652, which were filled in by treatment with the Klenow fragement of DNA polymerase I prior to ligation with pPX1651, were inserted in the same orientation. This procedure resulted in the insertion of an additional asparagine residue between the 16 amino acid blocks encoded by the oligonucleotide. Western blotting of cell extracts, utililizing monoclonal antibodies specific for the P. falciparum repeat region, demonstrated that these clones expressed immunoreactive proteins of an appropriate molecular weight for recombinant flagellin.

Detailed Description Paragraph Right (103):
A similar strategy was employed to create recombinant flagellin molecules expressing the repeated eight amino acid sequence of the P. berghei CS protein (FIG. 3B). In this set of experiments, oligonucleotides were ligated together prior to treatment with Klenow enzyme in order to ensure the insertion of contiguous oligonucleotides, all in the same orientation and without intervening DNA sequences. Clones were obtained containing one, two or three copies of the specific oligonucleotide, and were named pPX1661, pPX1662, and pPX1663, respectively. Bacteria harboring these clones were found to express immunoreactive proteins of the appropriate molecular weight when screened by western analysis utilizing a P. berghei CS-specific monoclonal antibody.

Detailed Description Paragraph Right (104):
A similar strategy was employed for the expression, as a flagellin fusion protein, of an epitope of an exotoxin of the pathogenic bacterium Vibrio cholerae. For the construction of this recombinant, complementary oligonucleotides (FIG. 4B) encoding the epitope represented by the CTP3 peptide (FIG. 4A) of the Cholera toxin B subunit (Jacob, C. O., et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:7611) were synthesized. The amino acid sequence encoded by the synthetic oligonucleotides was as follows:

Detailed Description Paragraph Right (105):
In order to express these recombinant flagellin molecules in an attenuated bacterial strain for use as live vaccines, all of the constructions described above (FIG. 5) were introduced into attenuated strains of S. dublin (SL1438 and SL5927) by phage tranduction (Schmeiger, 1972, Mol. Gen. Genetics 119:75). The method used to attenuate these strains has been described by Stocker and his coworkers (Hoiseth, S. K. and Stocker, B. A. D., 1981, Nature 291:238; Stocker, B. A. D., et al., 1982, Dev. Biol. Std. 53:47; U.S. Pat. No. 4,550,081). Specifically, deletions were introduced into the gene aroA, resulting in pleiotropic requirements for phenylalanine, tryptophan, tyrosine, the folic acid precursor p-aminobenzoic acid, and the enterochelin precursor, dihydroxybenzoic acid. p-aminobenzoic acid is absent from animal tissues, and members of the Enterobacteriaceae are unable to assimilate folic acid from animal tissues, resulting in their attenuation within an animal or human host. Western blot analysis was performed on extracts from each of these strains, and the synthesis of recombinant flagellins was demonstrated using both antibodies directed against flagellin epitopes (FIG. 6), indicating that these strains could be valuable as live vaccines to induce immune responses against the foreign epitopes inserted into the flagellin molecules.

Detailed Description Paragraph Right (106):
Exposure of the foreign epitope at the surface of the flagella was detected by gold immunolabeling of the flagella of Formalin-fixed bacteria, with MAb TE33 as the first antibody. Strain SL5676 harboring either plasmid pLS411, which has the complete CTP3 insert or plasmid pLS408, with the in vitro deletion but not the insert were labeled by treatment with MAb TE33 and gold-conjugated goat antibody to mouse IgG (Janssen) for electron microscope visualization (.times.30,000). Visualization of the label indicated that the CTP3 epitope was present on the surface of the bacteria.

Detailed Description Paragraph Right (107):
The ability of the recombinant flagellin proteins to polymerize into intact flagella and to therefore be present on the external surface of the bacteria was demonstrated by their restoration of motility in a normally non-motile (because flagellin-negative) host (Table III).

Detailed Description Paragraph Right (108):
In order to demonstrate the ability of recombinant flagellins to deliver foreign epitopes to the host immune system, C57BL/6 mice were immunized with partially purified flagella isolated from S. dublin SL1438 expressing in each flagellin molecule two copies of the P. berghei CS immunodominant repeat (encoded by plasmid pPX1661) or wild type H1-d flagella (encoded by plasmid pPX1650). Mice were injected subcutaneously with approximately 25 micrograms of flagellin protein emulsified in complete Freund's adjuvant at week 0 and boosted with 25 micrograms of the same preparation subcutaneously in incomplete Freund's adjuvant 4 weeks later. Animals were bled prior to the first and second immunizations and again two weeks after the booster. Sera were assayed by ELISA for antibodies specific for synthetic peptides encoding two copies of the P. berghei CS repeat (DPAPPNAN). Anti-P. berghei antibodies (FIG. 7) were slightly above background 4 weeks after the primary immunization, and levels increased dramatically following the booster imunization, whereas levels of these antibodies in animals immunized with control wildtype flagella (encoded by plasmid pPX1650) were not significantly different from prebleed values (FIG. 7, week 0).

Detailed Description Paragraph Right (109):
Immunization of C57BL/6 mice with live S. dublin SL1438 expressing recombinant flagella carrying the P. berghei CS epitope (encoded by plasmid pPX1662) also induced significant levels of serum antibodies to this epitope relative to control animals immunized with the same bacterial strain expressing wild-type H1-d flagella (encoded by plasmid pPX1650) (FIG. 8), illustrating the ability of live attenuated bacteria to deliver a foreign epitope as a flagellin fusion protein expressed on the surface of these organisms.

Detailed Description Paragraph Right (110):
For tests of immunogenicity, we replaced the phase-1 flagellin gene, H1-g,p of aromatic-dependent live-vaccine S. dublin strain SL1438 (Clements, J. et al., 1987, Infect. Immunol. 53:685; Dougan, G et al., 1987, Parasite Immunol. 9:151; and Poirier, T. P. et al., 1988, J. Exp. Med. 68:25) with a flagellin allele inactivated by a transposon, H1-i::Tn10; as S. dublin is monophasic, the resulting strain, SL5928, was nonmotile but became motile when transformed with plasmids containing either the wild-type, the deletion, or the chimeric form of H1-d, just as observed for the flagellin-negative S. typhimurium host, SL5676. The pUC-derived plasmids are stable in the live vaccine strain used, as shown by the ampicillin resistance of all of more than 100 colonies from a bacterial suspension after two passages in broth without ampicillin and by the ampicillin resistance of all colonies recovered from mouse livers at autopsy. We immunized C57BL/6 mice with three intraperitoneal injections of 5.times.10.sup.6 bacteria, either Formalin-killed or live, at 7-day intervals. A week after the last injection the mice were bled and their sera were tested by enzyme-linked immunosorbent assay (ELISA) for reactivity with CTP3 peptide or whole cholera toxin (FIG. 9). We detected antibody to the inserted epitope in all the sera; all sera reacted as strongly with cholera toxin as with the CTP3 peptide.

Detailed Description Paragraph Right (112):
We demonstrate the expression of epitopes critical to the induction of protective immune responses to pathogenic organisms, as fusion proteins with flagellin, the protein of bacterial flagellar filaments. Several recombinant flagellin genes were constructed which encoded epitopes normally expressed by a protozoan parasite, or by a bacterium. The immunodominant repeating epitope of the circumsporozoite (CS) protein of P. falciparum and the analogous epitope associated with P. berghei were inserted into a region of the H1-d gene of Salmonella muenchen. An oligonucleotide encoding a protective epitope present on the binding subunit of Cholera toxin (CT-B) was also inserted into an H1-d flagellin gene. All of these recombinant constructions were shown to express molecules which migrated through SDS-PAGE gels with mobilities consistent with their expected molecular weights. In addition, these molecules were recognized on Western blots by antisera specific for the H1-d flagellin molecule as well as by reagents which recognize the heterologous epitopes on the native protein.

Detailed Description Paragraph Right (113):
These hybrid proteins retain their ability to be expressed on the surface of recombinant bacteria thus facilitating isolation and purification of these molecules for use as components of a subunit vaccine. In addition, these molecules were not only expressed by E. coli harboring the recombinant plasmids, but were also introduced into several attenuated Salmonella strains which can be useful as live vaccines. Expression of recombinant flagellin molecules in attenuated, invasive bacteria, can allow the formulation of live vaccines against essentially any pathogen for which critical, immunogenic epitopes can be identified.

Detailed Description Paragraph Right (114):
In this study we inserted two specific HBV S gene sequences encoding respectively amino acid sequences S 122-137 and preS.sub.2 120-145 into the Salmonella flagellin gene H1-d and HBsAg epitopes were shown to be expressed by a flagellin-negative attenuated S. dublin strain transformed by the recombinant plasmids. Immunization of animals with live bacteria led to both anti-HBs and anti-flagellin responses.

Detailed Description Paragraph Right (116):
A polyclonal rabbit anti-H1-d (Salmonella Phase-1 flagellar antigen) serum, received from Dr. P. H. Makela, National Public Health Institute, Helsinki, Finland, was used as an anti-flagellin serum. Polyclonal goat anti-HBs (raised against native HBsAg purified from human plasma) was purchased from Dako company. The antisera against peptides S 122-137 and preS.sub.2 120-145 were raised by immunization of guinea pigs with the respective synthetic peptide conjugated with thyroglobulin. Optimal dilutions of these antisera established by titration were used to detect expression of the respective HBsAg epitopes in bacterial lysates by immunoblotting.

Detailed Description Paragraph Right (120):
Two synthetic oligonucleotides each encoding an HBsAg (subtype ayw) amino acid sequence that appears to contain a protective or partially protective epitope were used in this study (S 122-137 and preS.sub.2 120-145). The upper lines in FIG. 10 represent the nucleotide sequences of the corresponding synthetic oligonucleotides which were designed for insertion in-frame into the EcoRV sites of the flagellin gene (FIG. 10). The codons chosen were the most frequently used in the Salmonella flagellin gene H1-d (Wei, L.-N. and Joys, T. M., 1985, J. Mol. Biol. 186:791). Restriction sites for KpnI and BamHi (for the S 122-147 and preS.sub.2 120-145 coding sequences respectively) were included to allow identification of recombinants by restriction analysis. A half site for EcoRV was put at the 3' end of the preS.sub.2 oligonucleotide to facilitate ligation with oligonucleotides for other HBV epitopes. Two stop codons (underlined) were placed in the complementary strand for the preS.sub.2 oligonucleotide for easy selection of clones with inserts in the desired orientation. The flagellin gene was contained in plasmid pLS405 consisting of a 3.8 kB S. muenchen genome fragment containing the 1.5 Kb flagellin coding sequence cloned into the EcoRI site of plasmid pUC19 (see Example 1). The central hypervariable region of the wild-type flagellin gene contains two in-frame EcoRV sites with 48 base pairs (bp) apart (FIG. 10). Deletion of this EcoRV fragment in pLS405, to produce plasmid pLS408, reduces but does not abolish the flagellation of bacteria (see Example 1). Overlapping complementary single-stranded synthetic oligonucleotides were hybridized, phosphorylated, repaired with the Klenow fragment of the E. coli DNA polymerase to make blunt end double-stranded DNA fragments, then ligated into EcoRV site of pLS408 with T.sub.4 DNA ligase, and the ligation reaction mixture was used to transform CL447, a variant of the flagellin-negative strain E. coli C600 hag.sup.-. Clones with recombinant plasmids were identified by colony hybridization using the respective synthetic oligonucleotide labeled with .sup.32 P as probe and by restriction digestion. The number, orientation, reading frame and fidelity of inserts was determined by dideoxynucleotide sequencing (Sanger, F., et al., 1977 Proc. Natl. Acad. Sci. U.S.A. 74:5463), using a 15 nucleotide synthetic primer corresponding to a flagellin gene sequence about 30 bp downstream of the EcoRV site. Several recombinant plasmids with 1 to 3 copies of the respective synthetic oligonucleotide sequence in different orientations were isolated and further characterized.

Detailed Description Paragraph Right (121):
Recombinant plasmids to be further analyzed were used to transform S. typhimurium LB5000 (a restriction-negative, modification proficient and non-flagellated strain with mutation flaA66) competent cells and then transferred to a flagellin-negative live vaccine strain of S. dublin SL5928 by transduction using phage P22 HT105/1 int in each case with selection for ampicillin resistance. SL5928 is an aromatic-dependent strain derived from S. dublin SL1438 (Smith, B. P., et al., 1984, Amer. J. Veterin. Sci. 45:2231); it is non-motile because it is monophasic, with its single flagellin gene inactivated by transposon, Tn10, insertion.

Detailed Description Paragraph Right (122):
Antigens expressed in both the E. coli C600 hag.sup.- variant strain and S15928 were examined by immunblotting using either rabbit anti-H1-d (used as anti-flagellin), or goat anti-HBsAg or anti-synthetic preS.sub.2 120-145 peptide antisera. Bacterial lysates of the E. coli C600 hag.sup.- variant transformed with recombinant plasmids containing sequences encoding S 122-137 (S16e and S20e), sequence preS.sub.2 121-145 (ps8e) or sequence for preS.sub.2 120-145 (pS21e), and S. dublin SL5928 containing the same plasmids (S16s, S20s and pS8s, pS21s respectively) and controls consisting of lysates of the untransformed E. coli C600 hag.sup.- strain and S. dublin SL5928, S. dublin SL5985 which is SL5928 transformed with only the parent plasmid pLS405 with deletion of the EcORV fragment and HBsAg from a patient serum, were heated to 100.degree. C. for three minutes before loading. Proteins were separated by sodium dodecyl sulfate-12.5% polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, U. K., 1970, Nature 227:680). The proteins were transferred to nitrocellulose filters which were immunostained with either rabbit anti-flaggelin H1-d antiserum indicated as anti-F or goat anti-HBsAg or rabbit anti-synthetic peptide preS.sub.2 120-145. Incubation with an appropriate second antibody conjugated with either alkaline-phosphatase or peroxidase

was followed by reaction with NBT-BCIP (for alkaline-phosphatase) or DAB-H.sub.2 O.sub.2 (for peroxidase).

Detailed Description Paragraph Right (127):
The assembly of recombinant flagellin carrying HBsAg epitopes was investigated by electron microscopy of bacteria expressing the hybrid flagellin. In the motile clone, flagella with morphology indistinguishable from that of wild type flagella were seen on most bacteria. In the three non-motile (as judged by ability to spread on semisolid agar) but flagellin-positive clones S16, pS8, and pS21, very few flagella were observed (data not shown).

Detailed Description Paragraph Right (128):
FIG. 11 summarizes characteristics of seven recombinant plasmids that have been analysed. The solid (for S sequences) or hatched (for preS.sub.2 sequences) arrows represent the orientation and number of synthetic oligonucleotide sequences inserted between the EcoRV sites of the flagellin gene with respect to the orientation of the flagellin gene (represented by the stippled arrows) of pLS405. Bacterial motility was tested using semisolid agar. Rabbits were immunized with S. dublin SL5928 transformed with individual plasmids and the antibody responses were measured by ELISA using each synthetic peptide as coating antigen. "nd" represents "not done". As expected, only plasmids with S or preS.sub.2 coding sequences in the same orientation as the flagellin gene (S16, S20, pS8, and pS21) led to expression of hybrid flagellin proteins with detectable S or preS.sub.2 determinants. Among the small number of plasmids examined, three of the four with an insert in an orientation opposite that of the flagellin gene (S20, S6 and S27) were motile; lysates of bacteria carrying the fourth, pS2, did not bind anti-d antibody, as expected since the preS.sub.2 oligonucleotide in reverse orientation includes two termination codons (FIG. 10). Those with one or more inserts in the same orientation and no insert in the opposite orientation (S16, pS8 and pS21) did not spread in semisolid medium although scanty flagella were detected by electron microscopy and both flagellin and HBsAg epitopes were detected by immunoblotting.

Detailed Description Paragraph Right (129):
The immunogenicity of HBsAg epitopes in the hybrid flagellin proteins was first tested by intramuscular immunization of rabbits with live S. dublin SL5928 expressing hybrid flagellin (FIG. 12). FIG. 12 shows antibody responses of rabbits immunized intramuscularly with live S. dublin SL5928 transformed with S16 or pS21. Anti-flagellin indicates antibody detected by ELISA with the native flagellin protein purified from SL5928 transformed with the plasmid containing the wild type flagellin gene, anti-peptide represents antibodies detected by ELISA with synthetic peptides S 122-137 and preS.sub.2 120-145 (for animals immunized with SL5928 carrying S16 and pS21 respectively, and anti-HBs represents antibody detected by ELISA with HBsAg produced in CHO cells.

Detailed Description Paragraph Right (130):
High titers (above 10.sup.4) of anti-flagellin antibodies were elicited in the two animals receiving SL5928 transformed with plasmid S16 (with sequence encoding S 122-137) and in the two receiving SL5928 transformed with plasmid pS21 (with sequence encoding preS.sub.2 120-145). The ELISA titers of anti-peptide antibodies (anti-S 122-137 or anti-preS.sub.2 120-145 in the respective rabbits immunized with SL5928 carrying the corresponding plasmid) varied between 10.sup.3 and 2.times.10.sup.4 These antisera reacted with recombinant HBsAg (subtype ayw and containing preS.sub.2 sequence) produced in Chinese hamster ovary (CHO) cells (kindly provided by Dr. P. Toillais of Institut Pasteur) (Michel, M. K., et al., 1985, Biotechnology 3:561) with peak titers of approximately 6400 in two of four rabbits. The immune sera from these rabbits also reacted strongly with native HBsAg purified from HBv infected chimpanzees detected by Abbott Laboratory's Ausab assay (data not shown). Rabbits immunized with SL5928 transformed with plasmids S20 and pS8 respectively responded similarly (data not shown) to the animals immunized with SL5928 containing S16 and pS21. In two rabbits immunized with SL5928 containing the parental plasmid pLS405 without insertion of HBV sequences, high levels of anti-flagellin antibody were detected as expected,

and no anti-S or anti-preS.sub.2 peptide or anti-HBsAG antibodies were detected. None of the animals inoculated with this attenuated S. dublin mutant (SL5928) manifested signs of septic shock or other illness. These results indicate that the hybrid flagella expressed by S. dublin SL5928 carrying the recombinant plasmid contain HBsAg epitopes that are immunogenic and that antibody elicited by them reacts with plasma derived or recombinant HBsAg. .

Detailed Description Paragraph Right (131):
Synthetic peptides S122-137 and preS.sub.2 120-145 specifically blocked the binding of HBsAg produced in CHO cells by antibodies in the immune sera but not the preimmune sera (data not shown) of rabbits immunized with SL5928 clones expressing the S or preS.sub.2 epitopes respectively, confirming that the anti-HBs in these animals was directed at epitopes encoded by the sequences introduced into the flagellin gene.

Detailed Description Paragraph Right (132):
To determine whether anti-HBs responses would result from oral adminstration of live attenuated S. dublin SL5928 expressing hybrid flagella, experiments were carried out in rabbits, mice and guinea pigs. FIG. 13 shows anti-peptide and anti-HBs titers in mice after oral vaccination with SL5928 transformed with each of the recombinant plasmids S16, S20, pS8 and pS21 and with the unaltered flagellin gene pLS405. Significant titers of the respective anti-peptide and anti-HBs were detected in all animals although the titers were lower than those observed after intramuscular immunization of rabbits. Oral adminstration of pLS405 transformed bacteria SL5928 resulted in no detectable anti-HBs or anti-peptide antibody as expected. The titers of anti-peptide and anti-HBs in rabbits and guinea pigs (data not shown) were similar (80 to 640) to those in mice (FIG. 13) after oral administration of live S. dublin SL5928. No diarrhea or other disease manifestations were observed in any animal given S. dublin SL5928 orally. These experiments indicate that immune responses to HBsAg epitopes are elicited by oral vaccination with live S. dubin SL5928 expressing hybrid flagella.

Detailed Description Paragraph Right (133):
In this Example, we have shown that nucleotide sequences encoding antigenic regions of HBsAg polypeptides can be inserted into the hypervariable region of Salmonella flagellin gene and these genes in an attenuated Salmonella mutant can be expressed. Some resulting hybrid flagellin proteins can be assembled into functional flagella as tested by ability to spread in semisolid medium; other hybrid flagellins were not assembled into filaments, except perhaps in a small minority of bacteria. The hybrid flagella contain both flagellin and HBsAg epitopes detected by immunoblotting. The HBsAg epitopes were detected with antisera raised against specific synthetic peptides and against serum-derived HBsAg. Clearly, the number and orientation of HBsAg sequences inserted into the flagellin gene affected the ability of the protein to be assembled into functional flagella. Interestingly, a HBsAg sequence inserted in the same (and not in the opposite) orientation as the flagellin gene reduced bacterial motility suggesting that the specific viral envelope protein sequence (S 122-137) replacing a natural flagellin sequence of the same size significantly altered the conformation of the hybrid flagellin. In addition, replacing the 16 amino acid flagellin deletion with a 27 amino acid insert (preS.sub.2 120-145) did not prevent expression of flagellin but affected its function. In both, HBsAg epitopes recognized by antisera to native HBsAg were detected in the hybrid flagellin protein. These sequences as presented by live bacteria were immunogenic and elicited antibody that recognized native HBsAg. Thus, flagellin represents a bacterial protein in which viral antigens can be presented in a form that is immunogenic in live strains of Salmonella.

Detailed Description Paragraph Right (141):
To construct hybrid flagellin gene encoding epitope of the rotavirus VP7 (AA 275-292), synthetic oligonucleotides representing amino acids 275-292 of the rotavirus VP7 with the following sequence were inserted into the flagellin expression vector pPX1651 (see Example 1):

Detailed Description Paragraph Right (142):

Following transformation, recombinants were screened for insertion of the epitope by restriction enzyme mapping, western blotting and nucleotide sequencing. The resulting recombinant plasmid, pROTA92-19, was introduced into Salmonella dublin SL5927, and recombinant flagella prepared as described previously.

Detailed Description Paragraph Right (143):
The ability of flagellin and flagellin with the rotavirus 275-292 epitope (determined from the VP7 bovine rotavirus sequence as described above) to compete with infectious rotavirus for MA-104 cell receptors was determined. The virus stock used for the competition study was bovine rotavirus strain C486, which was activated with 50 ug trypsin per ml. An appropriate dilution of this stock was used in the competition experiment such that the final number of plaque forming units was 30-50. The initial concentration of the stock flagellin preparation, used as a control, was 3.55 mg/ml, while the stock preparation of flagellin containing the 275-292 epitope was at a concentration of 2.0 mg/ml. Appropriate dilutions of these preparations were made such that the final flagellin concentration was 1.25 ug, 25 ug, 50 ug, or 100 ug per 1.times.10.sup.5 cells.

Detailed Description Paragraph Right (145):
Each assay was carried out in triplicate and the flagellin or flagellin containing the 275-292 epitope preparations were also used alone on cell monolayers at the indicated concentrations to control for any adverse effect of the peptides themselves on cell monolayers.

Detailed Description Paragraph Right (146):
Delivery of certain immunogenic epitopes may result in the induction of specific cellular immune responses such as cell proliferation, elaboration of cytokines and specific lysis of target cells expressing those epitopes. In order to demonstrate the capacity of recombinant flagella to induce cellular immune responses, a predicted and experimentally confirmed T cell epitope was employed as a model for these experiments. The epitope which was chosen is comprised of amino acids 366-383 of the CRM197 protein (a

Detailed Description Paragraph Right (149):
These oligonucleotides were subcloned into the flagellin expression plasmid pPX1647. This plasmid is a modification of the original vector pPX1651 where the single Bam HI restriction site has been destroyed by cutting, creating flush ends by treatment with Klenow enzyme, and religating, and into which the following oligonucleotide was inserted at the unique EcoRV site:

Detailed Description Paragraph Right (150):
The underlined codons represent three separate restriction sites, EcoRV, ClaI and BamHI, respectively. This insertion results in the introduction of three unique restriction enzyme recognition sites which facilitate subsequent insertion of sequences encoding foreign epitopes. Plasmid pPX1647 was digested with EcoRV and BamHI, and religated in the presence of an excess amount of the oligonucleotide fragments encoding the CRM197 epitope. Following transformation, recombinants were isolated and characterized by restriction enzyme mapping, western blotting and nucleotide sequencing. The resulting recombinant plasmid, pCRM7F, was introduced into Salmonella dublin SL5927, and recombinant flagella prepared as described in Example 1.

Detailed Description Paragraph Right (151):
50 ug of the purified recombinant flagellin preparation was emulsified in an equal volume of complete Freund's adjuvant, and administered to SJL mice s.c. at the base of the tail. As controls, other groups of SJL mice were immunized in a similar fashion with non-recombinant flagella (1650) and purified CRM197 protein, as described in U.S. patent application Ser. No. 07/150,688, filed Feb. 1, 1989.

Detailed Description Paragraph Right (153):
FIG. 14 shows data generated when lymph node cells of SJL mice were primed with recombinant flagella. SJL

mice were immunized with 50ug of purified CRM197 protein (.DELTA.), recombinant flagella encoding the CRM197 366-383 epitope (.diamond-solid.), or purified wild type (1650) flagella (.box-solid.) in complete Freund's adjuvant s.c. at the base of the tail. Seven days post priming, lymph nodes were removed and single cell suspensions obtained. 3.times.10.sup.5 lymph node cells (LNC) were incubated with serial five fold dilutions of purified synthetic peptide encoding amino acids 366-383 of the CRM197 protein. Cells were cultured in RPMI 1640 containing 1% normal mouse serum at 37.degree. C. for three days, pulsed with 1.0 uCi per well of tritiated thymidine for 16 hours, and harvested for liquid scintillation counting. Data is presented as stimulation index (SI) vs. concentration of stimulating antigen where SI=cpm measured in wells in the presence of stimulating antigen divided by cpm in wells in the absence of any stimulating antigen. Each data point represents the mean and standard deviation of triplicate cultures.

Detailed Description Paragraph Right (154):
FIG. 15 presents data when the same lymph node cells were stimulated with purified CRM197 protein. SJL mice were immunized with 50 ug of purified CRM197 protein (.DELTA.), recombinant flagella encoding the CRM197 366-383 epitope (.diamond-solid.), or purified wild type (1650) flagella (.box-solid.) in complete Freund's adjuvant s.c. at the base of the tail; under conditions as described for data obtained in FIG. 14.

Detailed Description Paragraph Right (155):
The following Tables V and VI summarize the results obtained from motility studies, Western blot analysis and immunization studies using recombinant flagellin fusion proteins in various hosts. The methods for each of the tests summarized below are described in the previous Examples.

Detailed Description Paragraph Left (2):
Isolation of Sequences Encoding Immunogenic Epitopes for Expression as Recombinant Flagellins

Detailed Description Paragraph Left (3):
Construction of Recombinant Flagellin Genes

Detailed Description Paragraph Left (6):
Determination of Immunopotency of the Heterologous Epitope(s) Expressed as a Recombinant Flagellin

Detailed Description Paragraph Left (10):
Uses of Antibodies Directed Against Recombinant Flagellin

Detailed Description Paragraph Left (13):
Expression of Heterologous Epitopes as Recombinant Flagellin Fusion Proteins

Detailed Description Paragraph Left (25):
Partial Purification of Recombinant Flagella

Detailed Description Paragraph Left (29):
Construction of Recombinant Flagellin Genes

Detailed Description Paragraph Left (30):
Construction of a Recombinant Flagellin Gene Which Encodes an Epitope of a Malaria Parasite as a Flagellin Fusion Protein

Detailed Description Paragraph Left (31):
Construction of a Recombinant Flagellin Gene Which Encodes an Epitope of Cholera Toxin B Subunit as a Flagellin Fusion Protein

Detailed Description Paragraph Left (33):
Expression of Recombinant Flagellin Fusion Proteins

Detailed Description Paragraph Left (34):
Immunogold Labeling of Recombinant Flagellin

Detailed Description Paragraph Left (35):
Recombinant Flagellin Fusion Proteins are able to Assemble into Functional Flagella

Detailed Description Paragraph Left (36):
SL5927 is a non-motile derivative of SL1438 constructed by interrupting the chromosomal copy of the structural gene encoding the H1 antigen (flagellin) by insertion of a transposable element (Tn10); this strain, like other S. dublin, has no H2 allele. SL5927 is constructed as described above in the section entitled "Construction of Flagellin Minus Vaccine Strains". Introduction of any of the recombinant flagellin plasmids restores at least partial motility to this strain (Table III), indicating that these recombinant flagellins can polymerize into functional flagella, and that the foreign epitopes are therefore present on the external surface of the cell.

Detailed Description Paragraph Left (37):
Immunogenicity of the Heterologous Epitopes on Recombinant Flagellin Fusion Proteins

Detailed Description Paragraph Left (39):
Expression of Epitopes of Hepatitis B Surface Antigen as Recombinant Flagellin Fusion Proteins

Detailed Description Paragraph Left (49):
Detection of Epitope in Flagellin Construction

Detailed Description Paragraph Left (51):
Induction of Cellular Immune Responses With Hybrid Flagella Expressing Epitopes of CRM197

Detailed Description Paragraph Type 1 (2):
b. isolation of sequences encoding immunogenic epitopes for expression as recombinant flagellins;

Detailed Description Paragraph Type 1 (3):
c. construction of recombinant flagellin genes;

Detailed Description Paragraph Type 1 (5):
e. determination of immunopotency of the heterologous epitope(s) expressed as a recombinant flagellin; and

Detailed Description Paragraph Table (1):
TABLE I _____ HETEROLOGOUS ORGANISMS FROM WHICH DNA CAN BE ISOLATED FOR CONSTRUCTION OF GENES ENCODING FLAGELLIN FUSION PROTEINS _____ PARASITES: Plasmodium spp. Eimeria spp. Schistosoma spp. Trypanosoma spp. Babesia spp. Leishmania spp. Cryptosporidia spp. Toxoplasma spp. Pneumocystis spp. BACTERIA: Vibrio cholerae Streptococcus pyogenes Neisseria menigitidis Neisseria gonorrhoeae Corynebacteria diphtheriae Clostridium tetani Branhamella catarrhalis Bordetella pertussis Haemophilus spp. (e.g., influenzae) Chlamydia spp. Enterotoxigenic Escherichia coli VIRUSES: Human Immunodeficiency virus, type I Human Immunodeficiency virus, type II Simian Immunodeficiency virus Human T lymphocyte virus, type I, II and III Respiratory syncytial virus Hepatitis A virus Hepatitis B virus Hepatitis C virus Non-A, Non-B

Hepatitis Virus Herpes simplex virus, type I Herpes simplex virus, type II Cytomegalovirus Influenza virus Parainfluenza virus Poliovirus Rotavirus Coronavirus Rubella virus Measles virus Mumps virus Varicella Epstein Barr virus Adenovirus Papilloma virus Yellow Fever virus FUNGI: Candida spp. (especially albicans) Cryptococcus spp. (especially neoformans) Blastomyces spp. (dermatitidis) Histoplasma spp. (especially capsulatum) Coccidioides spp. (especially immitis) Paracoccidioides spp. (especially brasiliensis) Aspergillus spp.

Detailed Description Paragraph Table (2):

TABLE II ___ SALMONELLA SPECIES WHICH, IN ATTENUATED FORMS, CAN BE USED IN THE VACCINE FORMULATIONS OF THE PRESENT INVENTION* ___ S. typhi S. typhimurium S. paratyphi A S. paratyphi B S. enteritidis (e.g., serotype dublin) ___ *For a complete description of Salmonella serotypes, see Edwards and Ewing, 1986, Classification of the Enterobacteriaceae, 4th ed., Elsevier, N.Y.

Detailed Description Paragraph Table (4):

pPX1661* P. berghei CS protein 21 pPX1662* P. berghei CS protein 13.5 ppX1663* P. berghei CD protein 12 pLS411 cholera toxin B subunit 4.5 pUC18 -- 0 ___ .sup.1 (nonmotile) S. dublin SL5927 = S. dublin SL1438 H1i::Tn10 .sup.2 The native antigen, a portion of which is expressed as a recombinant flagellin fusion protein encoded by the plasmid at left. .sup.3 Overnight cultures were stabbed into the center of 60 mm petri dishes containing 0.3% agar in LB medium supplemented with 100 ug/ml ampicillin. Plates were incubated for 16 hours at room temperature and fo 6 hours at 37.degree. C. The diameter of the zone of bacterial spread in millimeters was then measured. *Encoding at least a portion of the flagellin H1d gene.

Detailed Description Paragraph Table (9):

TABLE V

Flagellin-Plasmids with Epitope-Specifying Inserts AA Lysate of Host-Plasmid Combinations Residues Plasmid LB5000/ Western Vaccine Origin Specified Number pLS Host.sup.a Combination Motility Blot Trial ___ Cholera CTP3= PLS411 G2615 SL1338 SL5938 n.a. + no toxin B 50-64 SL5676 SL5920 +, b + no subunit SL5928 SL5929 +, b + yes SL3261 SL5939 n.a. no Hepatitis B 122-137 S16= G2721 SL5676 SL5932 - S -122-137 pLS414 SL5928 SL5934 - + yes Protein Hepatitis B 122-137 S20= G2624 SL5676 SL5924 + S -137.sup.1 pLS413 SL5928 SL5933 + + yes Protein -122.sup.1 Hepatitis B pS121- pS8= SL5928 - + yes preS 145 pLS429 Hepatitis B pS120- pS21= SL5928 - + yes preS 145 pLS428 Hepatitis B pS145.sup.1 = pS2 SL5928 - - no preS (stop)-120' HIV Kennedy.sup.d pLS435 G2774 SL5928 SL7123 +, b + yes Envelope peptide Protein Strepto- AVTRGIND- pLS439 G2778 SL5928 SL5727 +, b + + coccus PQRAKEI type 5 M Protein ___ a

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SL1438 and SL3261 are S. dublin and S. typhimurium, respectively, both aroA but motile, so that ability of plasmid to cause production of flagella was not testable in them. b For these combinations, antipeptide antibody was shown to immobilize. c.sup.1 137.sup.1 -122.sup.1 indicates the amino acids at sites 122 to 137, which are specified by a DNA sequence for amino acids 122 to 137, inserted in reverse. d Sequence of HIV gp160 "Kennedy" peptide: ##STR1## Kennedy et al., 1986, Science 231:1556-59. Ratner et al., 1985, Nature (London) 313:277-284.

Detailed Description Paragraph Table (12):

___ Accession Bacterial Strain Plasmids Number

___ Salmonella dublin pPX1650: encoding the 67685 SL1438 full-length H1-d flagellin structural gene of S. muenchen Salmonella dublin pPX1653: encoding 4 67688

SL1438 copies the 4 amind acid repeat sequence of the sequence of the Plasmodium falciparum circumsporozoite protein as a recombinant fusion protein with H1-d flagellin Salmonella dublin pPX1662: encoding 4 67687 SL1438 copies of the 9 amino acid repeat sequence of the Plasmodium berghei circumsporozoite protein with H1-d flagellin Salmonella dublin pLS411: encoding the CTP3 67686 5L1438 peptide of the Cholera toxin B subunit as a recombinant fusion protein with H1-d flagellin Salmonella dublin no plasmid (vaccine strain 67944 SL5927 with Tn10 transposon inserted into H1 locus of Salmonella dublin SL5927) Salmonella dublin pROTA92-19: encoding 67945 SL5927 amino acids 275-292 of the Rotavirus VP7 as a recombinant fusion protein with H1-d flagellin _____

CLAIMS:

1. A recombinant gene comprising a nuclcotide sequence which encodes a flagellin fusion protein, which protein comprises a flagellin sequence containing a first epitope of a Salmonella H1-d flagellin structural gene with at least one epitope of a heterologous organism inserted within the flagellin sequence, wherein the flagellin protein is capable of binding to an antiflagellin antibody, wherein the DNA encoding at least one epitope of the heterologous organism is inserted in place of the DNA which naturally occurs between the natural EcoRV sites of the Salmonella H1-d gene.

☐ 5. Document ID: US 6129917 A

L1: Entry 5 of 16 File: USPT Oct 10, 2000

DOCUMENT-IDENTIFIER: US 6129917 A
TITLE: Immunogenic compositions comprising porphyromonas gingivalis proteins and/or peptides and methods

Detailed Description Paragraph Right (37):
Alternatively, mucosal immunity can be triggered by the administration to mucosal surfaces, for example, orally, of recombinant avirulent bacterial cells which express a protective epitope derived from a P. gingivalis protease, for example, RGP-1, HMW RGP or RGP-2, of particular interest is the expression of at least about 15 amino acids from the N-terminus of the RGP-2 or the N-terminus of a catalytic subunit of HMW RGP or HMW KGP. Avirulent Salmonella typhi and avirulent Salmonella typhimurium strains, suitable vectors and suitable promoters for driving expression are known to the art. The protective epitopes are advantageously expressed as fusions with other proteins, such as Salmonella flagellin, tetanus toxin fragment C, and E. coli LamB or MalE.

☐ 6. Document ID: US 6077515 A

L1: Entry 6 of 16 File: USPT Jun 20, 2000

DOCUMENT-IDENTIFIER: US 6077515 A
TITLE: Flagella-less borrelia

Brief Summary Paragraph Right (16):
In another embodiment, the immunoassay may comprise what is known to those of skill in the art as a competitive immunoassay; in such an assay binding is detected by adding a preparation of labeled antibodies reactive with a selected flagella-less Borrelia strain to the contacted sample and measuring binding of the labeled antibody to the antigenic preparation. Because antibodies in the sample will compete with the labeled antibodies for antigenic epitopes in the antigen preparation, binding of the labeled antibody will be inversely proportional to the concentration of antibody in the sample.

Drawing Description Paragraph Right (1):
FIG. 1: The oligonucleotide 5'-GCCAGCAGCATCATCAGAAG-3' SEQ. ID. NO. 3, which represented a conserved sequence of fla genes of two other strains of B. burgdorferi (Gassmann, et al., 1989), was synthesized and used to identify a flagellin gene-bearing clone in a library of genomic DNA of strain HB19 of B. burgdorferi in .lambda.FIX II. A 5.0 kb Bgl II fragment containing the complete fla gene of strain HB19 was subcloned into the plasmid vector pBR322 to yield recombinant plasmid pACA1. The nucleotide sequence of both strands of the flagellin gene and its 5' and 3' flanking sequences in pACA1 were determined by primer-directed sequencing of double-stranded pACA1 plasmid DNA. The start of transcription of flagellin gene was identified by primer extension analysis of total RNA isolated from strain HB19 B. burgdorferi. The analysis revealed the following: (i) the coding region for the flagellin gene of strain HB19 from positions 58-1065; (ii) the transcriptional start site, the C at position +1, 57 bp distant from the start codon; (iii) the likely ribosomal binding site (RBS) as GGAGG at position 45 to 49; and (iv) the likely "-10" (GCTATT) and "-35" (CGTT) promoter boxes. The numbers in the top column refer to nucleotides, those in the bottom column to amino acids. See SEQ. ID. No. 1.

Detailed Description Paragraph Right (14):
In some circumstances, it may be desirable to mutagenize the starting population of B. burgdorferi, for example, with chemicals, such as nitrosoguanidine, or irradiation, such as gamma-rays, in order to increase the frequency of mutation. The mutagenized B. burgdorferi are then selected by cloning by limiting dilution or by colony formation as described above. Another procedure which could be used comprises transposon mutagenesis of the flagellin gene followed by selection in antibiotic-containing BSK medium without Yeastolate (BSK I), and further selection for flagella-less mutants as described above. With the aid of the present disclosure, one may also devise methods for preparing flagella-less strains by recombinant DNA technology, for example, in vitro mutagenesis of the cloned flagellin gene and transformation of the mutant gene back into the borrelia. The sequence of the cloned flagellin gene and the 3' and 5' flanking sequences is shown in FIG. 1. The cloned flagellin gene could be accompanied by an antibiotic selection marker to aid selection of transformants in broth medium or on solid medium. Antibiotic-resistant transformants would be examined as to flagella phenotype. With any of these procedures, the mutations may be in the gene itself or in the regulatory regions, such as the promoter or terminator, for the flagellin gene. More specifically, such

mutations can include deletion of the entire coding region of the gene or portions thereof, deletion or mutagenesis of the ribosomal binding sequence (RBS), deletion or mutagenesis of the -10 and -35 promoter boxes, or insertion or deletion of DNA transcribed sequence of the gene such that a functional flagellar protein is not produced.

Detailed Description Paragraph Type 0 (5):
5. A. G. Barbour et al., A Borrelia-specific monoclonal antibody binds to a flagellar epitope, Infect. Immun. 1986; 52:549-54.

Detailed Description Paragraph Type 0 (48):
48. F. Sadallah et al., Production of specific monoclonal antibodies to Salmonella typhi flagellin and possible application to immunodiagnosis of typhoid fever, J. Inf. Dis. 1990; 161:59-64.

☐ 7. Document ID: US 6017536 A

L1: Entry 7 of 16 File: USPT Jan 25, 2000

DOCUMENT-IDENTIFIER: US 6017536 A
TITLE: Simian immunodeficiency virus peptides with antifusogenic and antiviral activities

Detailed Description Paragraph Table Type 3 (39):
TABLE VIII - 107 .times. 178 .times. 4 SEARCH MOTIF RESULTS SUMMARY FOR ALL PROCARYOTIC PROTEINS PCGENE 107 .times. 178 .times. 4 Prokaryotic Sequences AREA AREA AREA AREA AREA FILE NAME PROTEIN ORGANISM AREA 1 AREA 2 AREA 3 AREA 4 5 6 7 8 9 P120K.sub.-- RICRI 120 KD SURFACE-EXPOSED PROTEIN RICKETTSIA RICKETTSII 83-110 240-298 355-382 638-672 746- 1168-838 1202 P17K.sub.-- RICTY 17 KD ANTIGEN PRECURSOR RICKETTSIA TYPHI 67-94 P190K.sub.-- RICRI 190 KD ANTIGEN PRECURSOR (CELL SURFACE) RICKETTSIA RICKETTSII 241-268 460-487 607-634 754-781 829- 904- 1220- 1544- 1723- 856 931 1254 1571 1750 2065- 2131- 2096 2168 P22KD.sub.-- DESMO 22.6 KD PROTEIN DESULFUROCOCCUS MOBILIS 25-52 59-89 120-147 P40KD.sub.-- VIBAN 40 KD PROTEIN PRECURSOR VIBRIO ANGUILLARUM 153-196 P60IM.sub.-- ECOLI 60 KD INNER-MEMBRANE PROTEIN ESCHERICHIA COLI 511-538 P60IM.sub.-- PROMI 60 KD INNER-MEMBRANE PROTEIN PROTEUS MIRABILIS 14-41 P65KD.sub.-- ZYMMO 65 KD PROTEIN ZYMOMONAS MOBILIS 95-122 44-524 P6PGD.sub.-- BACSU PROB 6-PHOSPHOGLUCONATE DEHYDROGENASE BACILLUS SUBTILIS 24-51 218-246 P6PGD.sub.-- ECOLI 6-PHOSPHOGLUCONATE DEHYDROGENASE ESCHERICHIA COLI 205-232 P6PGD.sub.-- SALTY 6-PHOSPHOGLUCONATE DEHYDROGENASE SALMONELLA TYPHIMURIUM 205-232 PAACA.sub.-- STAAU 6'-AMINOGLYCOSIDE N-ACETYLTRANSFERASE STAPHYLOCOCCUS AUREUS 450-477 PAAT.sub.-- BACSP ASPARTATE AMINOTRANSFERASE BACILLUS SP 146-173 185-212 PAAT.sub.-- ECOLI ASPARTATE AMINOTRANSFERASE ESCHERICHIA COLI 351-378 PABC.sub.-- ECOLI ABC PROTEIN ESCHERICHIA COLI 176-203 PABIC.sub.-- LACLA ABORTIVE PHAGE RESISTANCE PROTEIN ABIC LOCTOCOCCUS LACTIS 85-126 170-204 209-273 PACCR.sub.-- AGRTU

TRANSCRIPTIONAL REPRESSOR ACCR AGROBACTERIUM TUMEFACIENS 127-154 PACEA.sub.-- ECOLI ISOCITRATE LYASE ESCHERICHIA COLI 398-432 PACON.sub.-- BACSU ACONITATE HYDRATASE BACILLUS SUBTILIS 48-75 PACON.sub.-- ECOLI ACONITATE HYDRATASE ESCHERICHIA COLI 41-68 613-640 PACOR.sub.-- ALCEU ACETOIN CATABOLISM REG PRO ALCALIGENES EUTROPHUS 85-112 PACP.sub.-- ECOLI ACYL CARRIER PROTEIN ESCHERICHIA COLI 4-31 PACRA.sub.-- ECOLI ACRIFLAVIN RESISTANCE PROTEIN A PRECURSOR ESCHERICHIA COLI 213-247 PACRB.sub.-- ECOLI ACRIFLAVIN RESISTANCE PROTEIN B ESCHERICHIA COLI 520-551 PACRF.sub.-- ECOLI ACRIFLAVIN RESISTANCE PROTEIN F ESCHERICHIA COLI 512-550 726-753 PACT3.sub.-- STRCO PUTATIVE KETOACYL REDUCTASE STREPTOMYCES COELICOLOR 157-184 PACTA.sub.-- LISMO ACTIN-ASSEMBLY INDUCING PROTEIN PRECURSOR LISTERIA MONOCYTOGENES 237-264 576-603 PACVS.sub.-- NOCLA ACV SYNTHETASE NOCARDIA LACTAMDURANS 3129-3163 PADAA.sub.-- BACSU METPHOSTRIESTER-DNA ALKYLTRANSFERASE BACILLUS SUBTILIS 136-170 PADDA.sub.-- BACSU ATP-DEPENDENT NUCLEASE SUBUNIT A BACILLUS SUBTILIS 398-425 454-481 522-556 1005- 1032 PADDB.sub.-- BACSU ATP-DEPENDENT NUCLEASE SUBUNIT B BACILLUS SUBTILIS 257-284 870-903 943-977 PADH1.sub.-- CLOAB NADPH-DEPENDENT BUTANOL DEHYDROGENASE CLOSTRIDIUM ACETOBUTYLICUM 284-311 PADHA.sub.-- CLOAB NADH-DEPENDENT BUTANOL DEHYDROGENASE A CLOSTRIDIUM ACETOBUTYLICUM 298-325 PADHB.sub.-- CLOAB NADH-DEPENDENT BUTANOL DEHYDROGENASE B CLOSTRIDIUM ACETOBUTYLICUM 298-325 PADHE.sub.-- CLOAB ALCOHOL DEHYDROGENASE CLOSTRIDIUM ACETOBUTYLICUM 653-680 770-806 PADHE.sub.-- ECOLI ALCOHOL DEHYDROGENASE ESCHERICHIA COLI 271-298 PADIY.sub.-- ECOLI PUTATIVE REGULATORY PROTEIN ADIY ESCHERICHIA COLI 45-72 PADP1.sub.-- MYCGE 140 KD ADHESION PRECURSOR MYCOPLASMA GENITALIUM 90-131 697-724 923-950 990- 1169- 1387- 1017 1199 1414 PADP1.sub.-- MYCPN ADHESION P1 PRECURSOR MYCOPLASMA PNEUMONIAE 1557- 1584 PADT.sub.-- RICPR ADP, ATP CARRIER PROTEIN RICKETTSIA PROWAZEKII 276-307 PAERA.sub.-- AERHY AEROLYSIN PRECURSOR AEROMONAS HYDROPHILA 278-305 PAGAL.sub.-- STRMU ALPHA-GALACTOSIDASE STREPTOCOCCUS MUTANS 419-483 597-633 PAGAR.sub.-- PSEAT BETA-AGARASE PRECURSOR PSEUDOMONAS ATLANTICA 26-53 PAGR.sub.-- STAAU ACCESSORY GENE REGULATOR PROTEIN STAPHYLOCOCCUS AUREUS 129-159 165-192 PAIL.sub.-- YEREN ATTACH INVAS LOCUS PROTEIN PRECURSOR YERSINIA ENTEROCOLITICA 19-46 PAK1H.sub.-- ECOLI ASPARTOKINASE I ESCHERICHIA COLI 3-30 466-493 503-530 PAK2H.sub.-- ECOLI ASPARTOKINASE II ESCHERICHIA COLI 51-78 608-635 PAK2.sub.-- BACSU ASPARTATE KINASE II ALPHA AND BETA SUBUNITS BACILLUS SUBTILIS 266-312 PAKAB.sub.-- CORGL ASPARTATE KINASE ALPHA AND BETA SUBUNITS CORYNEBACTERIUM GLUTMICUM 5-32 PALF.sub.-- ECOLI FRUCTOSE-BISPHOSPHATE ALDOLASE ESCHERICHIA COLI 286-316 PALGB.sub.-- PSEAE ALGINATE BIOSYN TRANSL REG PROTEIN ALGB PSEUDOMONAS AERUGINOSA 160-194 PALGE.sub.-- PSEAE ALGINATE PRODUCTION PROTEIN ALGE PRECURSOR PSEUDOMONAS AERUGINOSA 349-376 PALGP.sub.-- PSEAE TRANSCRIPTIONAL REGULATORY PROTEIN ALGP PSEUDOMONAS AERUGINOSA 81-115 PALKB.sub.-- PSEOL ALKANE-1 MONOOXYGENASE PSEUDOMONAS OLEOVORANS 115-142 PALKT.sub.-- PSEOL RUBREDOXIN-NAD(+) REDUCTASE PSEUDOMONAS OLEOVORANS 138-172 338-365 PALR2.sub.-- ECOLI ALANINE RACEMASE CATABOLIC PRECURSOR ESCHERICHIA COLI 9-36 PALR.sub.-- BACST ALANINE RACEMASE BACILLUS STEAROTHERMOPHILUS 326-353 PALSR.sub.-- BACSU ALS OPERON REGULATORY PROTEIN BACILLUS SUBTILIS 119-146 PALYS.sub.-- BACSP AUTOLYSIN PRECURSOR BACILLUS SP 157-187 PALYS.sub.-- BACSU AUTOLYSIN PRECURSOR BACILLUS SUBTILIS 147-191 PALYS.sub.-- STAAU AUTOLYSIN STAPHYLOCOCCUS AUREUS 244-271 PAMIA.sub.-- STRPN AMIA PROTEIN PRECURSOR STREPTOCOCCUS PNEUMONIAE 223-264 297-338 446-473 PAMID.sub.-- PSECL AMIDASE PSEUDOMONAS CHLORORAPHIS 72-99 PAMIE.sub.-- STRPN OLIGOPEPTIDE TRANSPORT PROTEIN AMIE STREPTOCOCCUS PNEUMONIAE 187-214 PAMPA.sub.-- ECOLI AMINOPEPTIDASE A/I ESCHERICHIA COLI 111-138 199-226 PAMPC.sub.-- SERMA BETA-LACTAMASE PRECURSOR SERRATIA MARCESCENS 231-258 PAMPL.sub.-- RICPR CYTOSOL AMINOPEPTIDASE RICKETTSIA PROWAZEKII 3-47 72-99 PAMPN.sub.-- ECOLI AMINOPEPTIDASE N ESCHERICHIA COLI 655-682 PAMPP.sub.-- ECOLI X-PRO AMINOPEPTIDASE ESCHERICHIA COLI 110-137 PAMPT.sub.--

THEAQ AMINOPEPTIDASE T THERMUS AQUATICUS 281-308 PAMY1.sub.-- DICTH ALPHA-AMYLASE 1 DICTYOGLOMUS THERMOPHILUM 507-534 PAMY2.sub.-- DICTH ALPHA-AMYLASE 2 DICTYOGLOMUS THERMOPHILUM 151-178 507-534 PAMY2.sub.-- SALTY CYTOPLASMIC ALPHA-AMYLASE SALMONELLA TYPHIMURIUM 70-104 PAMY3.sub.-- DICTH ALPHA-AMYLASE 3 DICTYOGLOMUS THERMOPHILUM 280-307 PAMYB.sub.-- BACCI BETA-AMYLASE PRECURSOR BACILLUS CIRCULANS 61-88 PAMYB.sub.-- BACPO BETA-AMYLASE BACILLUS POLYMYXA 60-87 266-293 1143- 1184 PAMYB.sub.-- CLOTU BETA-AMYLASE, THERMOPHILIC PRECURSOR CLOSTRIDIUM THERMOSULFUROGENES 269-296 378-405 459-486 PAMYG.sub.-- CLOSP GLUCOAMYLASE PRECURSOR CLOSTRIDIUM SP 103-148 480-510 PAMYM.sub.-- BACST MALTOGENIC ALPHA-AMYLASE PRECURSOR BACILLUS STEROTHERMOPHILUS 426-453 PAMYR.sub.-- BACS8 RAW-STARCH-DIGESTING AMYLASE BACILLUS SP 210-237 435-465 615-642 PAMY.sub.-- AERHY ALPHA-AMYLASE PRECURSOR AEROMONAS HYDROPHILA 415-453 PAMY.sub.-- ALTHA ALPHA-AMYLASE PRECURSOR ALTEROMONAS HALOPLANKTIS 166-913 PAMY.sub.-- BACAM ALPHA-AMYLASE PRECURSOR BACILLUS AMYLOLIQUEFACIENS 102-136 PAMY.sub.-- BACCI ALPHA-AMYLASE PRECURSOR BACILLUS CIRCULANS 212-239 437-474 PAMY.sub.-- BACME ALPHA-AMYLASE PRECURSOR BACILLUS MEGATERIUM 61-88 441-482 PAMY.sub.-- BACSU ALPHA-AMYLASE PRECURSOR BACILLUS SUBTILIS 165-205 281-308 PAMY.sub.-- BUTFI ALPHA-AMYLASE PRECURSOR BUTYRIVIBRIO FIBRISOLVENS 377-418 546-573 579-606 795-822 PAMY.sub.-- CLOAB PUTATIVE ALPHA-AMYLSE CLOSTRIDIUM ACETOBUTYLICUM 283-310 PAMY.sub.-- CLOTU ALPHA-AMYLASE PRECURSOR CLOSTRIDIUM THERMOSULFUROGENES 431-468 612-642 PAMY.sub.-- STRLM ALPHA-AMYLASE PRECURSOR STREPTOMYCES LIMOSUS 173-200 PANFA.sub.-- AZOVI NITROGEN FIXATION PROTEIN ANFA AZOTOBACTER VINELANDII 232-259 PANFD.sub.-- AZOVI NITROGENASE IRON--IRON PROTEIN ALPHA CHAIN AZOTOBACTER VINELANDII 95-122 PANFK.sub.-- AZOVI NITROGENASE IRON--IRON PROTEIN BETA CHAIN AZOTOBACTER VINELANDII 369-396 PANGR.sub.-- VIBAN ANGR PROTEIN VIBRIO ANGUILLARUM 93-120 169-203 PAPCE.sub.-- FREDI PHYCOBILISOME 120 KD LINKER POLYPEPTIDE FREMYELLA DIPLOSIPHON 51-78 PAPCE.sub.-- SYNP6 PHYCOBILISOME LINKER POLYPEPTIDE SYNECHOCOCCUS SP 37-64 585-615 PAPCE.sub.-- SNYP4 PHYCOBILISOME 120 KD LINKER POLYPEPTIDE SYNECHOCYSTIS SP 52-79 PAPHC.sub.-- SALTY ALKYL HYDROPEROXIDE REDUCTASE C22 PROTEIN SALMONELLA TYPHIMURIUM 62-89 PAPI.sub.-- ACHLY PROTEASE I PRECURSOR ACHROMOBACTER LYTICUS 478-505 PAPPC.sub.-- ECOLI PROBABLE CYTOCHROME OXIDASE SUBUNIT I ESCHERICHIA COLI 118-148 PAPRD.sub.-- PSEAE ALKALINE PROTEASE SECRETION PROTEIN APRD PSEUDOMONAS AERUGINOSA 416-450 PAPRE.sub.-- PSEAE ALKALINE PROTEASE SECRETION PROTEIN APRE PSEUDOMONAS AERUGINOSA 133-193 208-235 247-277 PAPT.sub.-- ECOLI ADENINE PHOSPHORIBOSYLTRANSFERASE ESCHERICHIA COLI 121-148 PAPU.sub.-- THEET ALPHA-AMYLASE-PULLULANASE PRECURSOR THERMOANAEROBACTER ETHANOLICUS 276-303 347-374 936-982 987- 1210- 1381- 1014 1254 1408 PARCA.sub.-- MYCAR ARGININE DEIMINASE MYCOPLASMA ARGININI 60-87 218-245 PARCB.sub.-- ECOLI AEROBIC RESPIRATION CONTROL PROTEIN ARCB ESCHERICHIA COLI 102-150 302-329 399-426 PARCD.sub.-- PSEAE PROBABLE ARGININE/ORNITHINE ANTIPORTER PSEUDOMONAS AERUGINOSA 274-301 386-420 PARGA.sub.-- ECOLI AMINO-ACID ACETYLTRANSFERASE ESCHERICHIA COLI 82-109 PARGT.sub.-- ECOLI LYS-ARG-ORN-BINDING PROTEIN (LAO) PRECURSO ESCHERICHIA COLI 84-111 PAROA.sub.-- STAAU PHOSPHOSHIKIMATE 1-CARBOXYVINYL TRANSFER STAPHYLOCOCCUS AUREUS 86-120 PAROC.sub.-- ECOLI CHORISMATE SYNTHASE ESCHERICHIA COLI 68-95 PAROC.sub.-- SALTI CHORISMATE SYNTHASE SALMONELLA TYPHI 68-95 PAROD.sub.-- BACSU DEHYDROQUINATE DEHYDRATASE BACILLUS SUBTILIS 49-76 PAROK.sub.-- ECOLI SHIKIMATE KINASE I ESCHERICHIA COLI 84-118 PARP4.sub.-- STRPY IGA RECEPTOR PRECURSOR STREPTOCOCCUS PYOGENES 12-46 127-157 266-324 PARP.sub.-- ECOLI ARP PROTEIN ESCHERICHIA COLI 255-282 PARSA.sub.-- ECOLI ARSENICAL PUMP-DRIVING ATPASE ESCHERICHIA COLI 201-238 PARSB.sub.-- ECOLI ARSENICAL PUMP MEMBRANE PROTEIN ESCHERICHIA COLI 291-318 PARSB.sub.-- STAAU ARSENICAL PUMP MEMBRANE PROTEIN STAPHYLOCOCCUS AUREUS 27-71 295-322 PARSB.sub.-- STAZY ARSENICAL PUMP MEMBRANE PROTEIN STAPHYLOCOCCUS XYLOSUS 27-71 295-322 PARSR.sub.-- STAAU ARSENICAL

RESIST OPERON REPRESSOR PROTEIN STAPHYLOCOCCUS AUREUS 56-93 PARTA.sub.-- ECOLI ARTA PROTEIN ESCHERICHIA COLI 3-30 PARTI.sub.-- ECOLI TRANSPORT SYSTEM PROTEIN ARTI ESCHERICHIA COLI 105-132 213-240 PARTP.sub.-- ECOLI TRANSPORT SYSTEM PROTEIN ARTP ESCHERICHIA COLI 176-206 PASA1.sub.-- ENTFA AGGREGATION SUBSTANCE PRECURSOR ENTEROCOCCUS FAECALIS 195-254 478-505 799-826 859-896 PASNA.sub.-- ECOLI ASPARTATE-AMMONIA LIGASE ESCHERICHIA COLI 127-158 PASNB.sub.-- ECOLI ASPARAGINE SYNTHETASE B ESCHERICHIA COLI 450-477 PASNC.sub.-- ECOLI REGULATORY PROTEIN ASNC ESCHERICHIA COLI 116-143

Detailed Description Paragraph Table Type 3 (45):
BACILLUS SUBTILIS 62-89 PFLGK.sub.-- SALTY FLAGELLAR HOOK-ASSOCIATED PROTEIN 1 SALMONELLA TYPHIMURIUM12-50 333-360 456-540 PFLGL.sub.-- ECOLI FLAGELLAR HOOK-ASSOCIATED PROTEIN 3 ESCHERICHIA COLI 61-105 229-266 PFLGL.sub.-- SALTY PFLHD.sub. -- ECOLI FLAGELLAR TRANSCRIPTIONAL ACTIVATOR FLHD ESCHERICHIA COLI 6-33 PFLIA.sub.-- PSEAE FLAGELLAR OPERON RNA POL SIGMA FACTOR PSEUDOMONAS AERUGINOSA 198-232 PFLIC.sub.-- ECOLI FLAGELLIN ESCHERICHIA COLI 3-41 186-23 295-329 431-466 PFLIC.sub.-- SALCH FLAGELLIN SALMONELLA CHOLERAE-SUIS 5-41 54-125 136-198 PFLIC.sub.-- SALMU FLAGELLIN SALMONELLA MUENCHEN 5-41 54-88 136-177 232-259 272- 376- 299 403 PFLIC.sub.-- SALPA FLAGELLIN SALMONELLA PARATYPHI-A 5-41 54-125 136-184 PFLIC.sub.-- SALRU FLAGELLIN SALMONELLA RUBISLAW 5-41 54-125 136-196 PFLIC.sub.-- SALTY FLAGELLIN SALMONELLA TYPHIMURIUM 5-41 54-125 136-200 PFLIC.sub.-- SERMA FLAGELLIN SERRATIA MARCESCENS 15-42 55-89 103-130 137- 275-164 321 PFLID.sub.-- ECOLI 216-298 386- 445 PFLID.sub.-- SALTY FLAGELLAR HOOK-ASSOCIATED PROTEIN 2 SALMONELLA TYPHIMURIUM 32-66 106-133 255-299 407-438 PFLIE.sub.-- BACSU FLAG HOOK-BASAL BODY PROTEIN FLIE BACILLUS SUBTILIS 8-35 PFLIF.sub.-- BACSU PFLIF.sub.-- SALTY FLAGELLAR M-RING PROTEIN SALMONELLA TYPHIMURIUM 484-529 PFLIG.sub.-- BACSU FLAGELLAR SWITCH PROTEIN FLIG BACILLUS SUBTILIS 35-62 PFLIG.sub.-- ECOLI FLAGELLAR SWITCH PROTEIN FLIG ESCHERICHIA COLI 44-71 PFLIH.sub.-- BACSU PROBABLE FLIH PROTEIN BACILLUS SUBTILIS 19-46 105-132 PFLIJ.sub.-- BACSU FLAGELLAR FLIJ PROTEIN BACILLUS SUBTILIS 7-37 PFLIJ.sub.-- SALTY FLAGELLAR FLIJ PROTEIN SALMONELLA TYPHIMURIUM 75-118 PFLIK.sub.-- BACSU PROBABLE FLIK PROTEIN BACILLUS SUBTILIS 77-104 117-144 PFLIL.sub.-- BACSU FLIL PROTEIN BACILLUS SUBTILIS 30-71 78-105 109-136 PFLIL.sub.-- ECOLI FLIL PROTEIN ESCHERICHIA COLI 105-132 PFLIL.sub.-- SALTY FLIL PROTEIN SALMONELLA TYPHIMURIUM 103-133 PFLIM.sub.-- BACSU FLIM PROTEIN BACILLUS SUBTILIS 148-175 PFLIM.sub.-- ECOLI FLIM PROTEIN ESCHERICHIA COLI 251-278 PFLIN.sub.-- CAUCR PROTEIN FLIT SALMONELLA TYPHIMURIUM 9-46 67-106 PFM12.sub.-- PSEAE FIMBRIAL PROTEIN PRECURSOR PSEUDOMONAS AERUGINOSA 30-67 80-114 PFM1A.sub.-- ECOLI TYPE-1 FIMBRIAL PROTEIN, A CHAIN PRECURSOR ESCHERICHIA COLI 5-32 PFM1C.sub.-- ECOLI TYPE-1 FIMBRIAL PROTEIN, C CHAIN PRECURSOR ESCHERICHIA COLI 11-38 PFM1.sub.-- ACTIV FIMBRIAL SUBUNIT TYPE 1 PRECURSOR ACTINOMYCES VISCOSUS 248-28 352-379 417-444 PFM98.sub.-- ECOLI FIMBRIAL PROTEIN 987P PRECURSOR ESCHERICHIA COLI 114-141 PFMA0.sub.-- BACNO FIMBRIAL PROTEIN PRECURSOR BACTEROIDES NODOSUS 110-137 PFMA1.sub.-- BACNO FIMBRIAL PROTEIN PRECURSOR BACTEROIDES NODOSUS 107-134 PFMA2.sub.-- BACNO FIMBRIAL PROTEIN PRECURSOR BACTEROIDES NODOSUS 107-134 PFMA7.sub.-- BACNO FIMBRIAL PROTEIN PRECURSOR BACTEROIDES NODOSUS 110-137 PFMAA.sub.-- BACNO FIMBRIAL PROTEIN PRECURSOR BACTEROIDES NODOSUS 123-150 PFMAF.sub.-- BACNO FIMBRIAL PROTEIN PRECURSOR BACTEROIDES NODOSUS 107-141 PFMAH.sub.-- BACNO FIMBRIAL PROTEIN PRECURSOR BACTEROIDES NODOSUS 95-122 PFMAI.sub.-- BACNO FIMBRIAL PROTEIN PRECURSOR BACTEROIDES NODOSUS 111-145 PFMAJ.sub.-- BACNO FIMBRIAL PROTEIN PRECURSOR BACTEROIDES NODOSUS 96-123 PFMCD.sub.-- PSEAE FIMBRIAL PROTEIN PRECURSOR PSEUDOMONAS AERUGINOSA 70-97 PFMDD.sub.-- BACNO POSSIBLE FIMBRIAL ASSEMBLY PRECURSOR FIMD BACTEROIDES NODOSUS 106-144 355-382 PFMDH.sub.-- BACNO POSSIBLE FIMBRIAL ASSEMBLY PRECURSOR FIMD BACTEROIDES NODOSUS 106-144 355-382

PFMF3.sub.-- ECOLI F17 FIMBRIAL PROTEIN PRECURSOR ESCHERICHIA COLI 97-124 PFMM1.sub.-- NEIME FIMBRIAL PROTEIN PRECURSOR NESSERIA MENINGITIDIS 70-97 PFMM2.sub.-- NEIGO FIMBRIAL PROTEIN PRECURSOR NESSERIA GONORRHOEAE 66-97 PFMM.sub.-- MORNO FIMBRIAL PROTEIN PRECURSOR MORAXELLA NONLIQUEFACIENS 108-146 PFMP1.sub.-- PSEAE FIMBRIAL PROTEIN PRECURSOR PSEUDOMONAS AERUGINOSA 30-67 80-114 PFMP3.sub.-- PSEAE FIMBRIAL PROTEIN PRECURSOR PSEUDOMONAS AERUGINOSA 70-97 PFMS1.sub.-- ECOLI CS1 FIMBRIAL SUBUNIT A PRECURSOR ESCHERICHIA COLI 60-87 112-139 PFMS3.sub.-- ECOLI CS3 FIMBRIAL SUBUNIT A PRECURSOR ESCHERICHIA COLI 49-98 PFM.sub.-- HAEIN MAJOR FIMBRIAL SUBUNIT PRECURSOR HAEMOPHILUS INFLUENZAE 102-129 PFNBA.sub.-- STAAU FIBRONECTIN-BINDING PROTEIN PRECURSOR STAPHYLOCOCCUS AUREUS 41-83 188-215 311-365 431-458 517- 652- 722- 555 686 756 PFOLC.sub.-- ECOLI FOLYLPOLYGLUTAMATE SYNTHASE ESCHERICHIA COLI 125-159 PFOLC.sub.-- LACCA FOLYLPOLYGLUTAMATE SYNTHASE LACTOBACILLUS CASEI 129-156 PFPG.sub.-- BACFI FORMAMIDOPYRIMIDINE-DNA GLYCOSYLASE BACILLUS FIRMUS 153-180 PFRDA.sub.-- ECOLI FUMARATE REDUCTASE FLAVOPROTEIN SUBUNIT ESCHERICHIA COLI 395-422 PRFDA.sub.-- WOLSU FUMARATE REDUCTASE FLAVOPROTEIN SUBUNIT WOLINELLA SUCCINOGENES 8-35 487-514 PFRZE.sub.-- MYXXA GLIDING MOTILITY REGULATORY PROTEIN MYXOCOCCUS XANTHUS 15-42 478-505 PFTHS.sub.-- CLOTH FORMATE-TETRAHYDROFOLATE LIGASE CLOSTRIDIUM THERMOACETUM 163-190 PFTR.sub.-- METTH FORMYLTRANSFERASE METHANOBACTERIUM 9-43 THERMOAUTOTROPHICU PFTSA.sub.-- VACSU CELL DIVISION PROTEIN FTSA BACILLUS SUBTILIS 76-110 PFTSA.sub.-- ECOLI CELL DIVISION PROTEIN FTSA ESCHERICHIA COLI 301-338 375-418 PFTSJ.sub.-- ECOLI CELL DIVISION PROTEIN FTSJ ESCHERICHIA COLI 4-31 PFTSL.sub.-- ECOLI CELL DIVISION PROTEIN FTSL ESCHERICHIA COLI 63-90 PFTSN.sub.-- ECOLI CELL DIVISION PROTEIN FTSN ESCHERICHIA COLI 151-188 PFTSX.sub.-- ECOLI CELL DIVISION PROTEIN FTSX ESCHERICHIA COLI 278-305 PFTSY.sub.-- ECOLI CELL DIVISION PROTEIN FTSY ESCHERICHIA COLI 230-260 PFUCR.sub.-- ECOLI L-FUCOSE OPERON ACTIVATOR ESCHERICHIA COLI 7-45 PFUMA.sub.-- BACST FUMARATE HYDRATASE CLASS I, AEROBIC BACILLUS STEAROTHERMOPHILUS 290-317 PFUMH.sub.-- BACSU FUMARATE HYDRATASE BACILLUS SUBTILIS 414-445 PFUR.sub.-- YERPE FERRIC UPTAKE REGULATION PROTEIN YERSINIA PESTIS 99-130 PG3P1.sub.-- ECOLI GLYC 3-PHOS DEHYDROGENASE A ESCHERICHIA COLI 302-329 PG3P2.sub.-- ANAVA GLYC 3-PHOS DEHYDROGENASE 2 ANABAENA VARIABLILIS 87-114 PG3P3.sub.-- ANAVA GLYC 3-PHOS DEHYDROGENASE 3 ANABAENA VARIABLILIS 162-189 PG3P3.sub.-- ECOLI GLYC 3-PHOS DEHYDROGENASE C ESCHERICHIA COLI 236-324 PG3P.sub.-- BACME GLYC 3-PHOS DEHYDROGENASE BACILLUS MEGATERIUM 49-76 237-271 PG3P.sub.-- BACSU GLYC 3-PHOS DEHYDROGENASE BACILLUS SUBTILIS 49-76 PG3P.sub.-- PYRWO GLYC 3-PHOS DEHYDROGENASE PYROCOCCUS WOESEI 259-286 PG3P.sub.-- THEMA GLYC 3-PHOS DEHYDROGENASE THERMOTOGA MARITIMA 290-328 PG6PB.sub.-- VACST GLUCOSE-6-PHOSPHATE ISOMERASE B BACILLUS STEAROTHERMOPHILUS 103-143 241-268 PG6PD.sub.-- ECOLI GLUCOSE-6-PHOSPHATE 1-DEHYDROGENASE ESCHERICHIA COLI 301-328 PG6PD.sub.-- ZYMMO GLUCOSE-6-PHOSPHATE 1-DEHYDROGENASE ZYMOMONAS MOBILIS 165-192 PGACA.sub.-- PSEFL CYANIDE CONTROL PROTEIN PSEUDOMONAS FLUORESCENS 178-205 PGAL1.sub.-- SALTY GALACTOKINASE SALMONELLA TYPHIMURIUM 86-113 PGAL7.sub.-- HAEIN GAL-1-PHOS URIDYLYLTRANSFERASE HAEMOPHILUS INFLUENZAE 124-158 239-269 PGAL7.sub.-- LACHE GAL-1-PHOS URIDYLYLTRANSFERASE LACTOBACILLUS HELVETICUS 304-338 PGALF.sub.-- SALTY GALACTOSE OPERON REPRESSOR SALMONELLA TYPHIMURIUM 53-91 PGALR.sub.-- HAEIN GALACTOSE OPERON REPRESSOR HAEMOPHILUS INFLUENZAE 182-209 PGAL.sub.-- PSEFL DE D-GALACTOSE 1-DEHYDROGENASE PSEUDOMONAS FLUORESCENS 251-278 PGCH2.sub.-- ECOLI GTP CYCLOHYDROLASE II ESCHERICHIA COLI 78-105 PGCH2.sub.-- PHOLE GTP CYCLOHYDROLASE II PHOTOBACTERIUM LEIOGNATHI 197-227 246-273 PGCSH.sub.-- ECOLI GLYCINE CLEAVAGE SYSTEM H PROTEIN ESCHERICHIA COLI 10-37 PGCSP.sub.-- ECOLI GLYCINE DEHYDROGENASE ESCHERICHIA COLI 216-246 PGCVA.sub.-- ECOLI GLYCINE CLEAVAGE SYSTEM TRANSACTIVATOR ESCHERICHIA COLI 60-94 PGENK.sub.-- ECOLI PROTEIN K ESCHERICHIA COLI 24-51 PGER1.sub.-- BACSU SPORE GERMINATION PROTEIN I BACILLUS

SUBTILIS 49-83 182-216 350-384 PGER3.sub.-- BACSU SPORE GERMINATION PROTEIN III PRECURSOR BACILLUS SUBTILIS 293-323 PGERE.sub.-- BACSU GERMINATION PROTEIN GERE BACILLUS SUBTILIS 13-40 PGGI2.sub.-- STAHA ANTIBACTERIAL PROTEIN 2 STAPHYLOCOCCUS HAEMOLYTICUS 6-33 PGGI3.sub.-- STAHA ANTIBACTERIAL PROTEIN 3 STAPHYLOCOCCUS HAEMOLYTICUS 6-33 PGIDA.sub.-- BACSU GLUCOSE INHIBITED DIVISION PROTEIN A BACILLUS SUBTILIS 396-423 PGIDA.sub.-- ECOLI GLUCOSE INHIBITED DIVISION PROTEIN A ESCHERICHIA COLI 533-568 PGIDA.sub.-- PSEPU GLUCOSE INHIBITED DIVISION PROTEIN A PSEUDOMONAS PUTIDA 539-566 PGIDB.sub.-- BACSU GLUCOSE INHIBITED DIVISION PROTEIN B BACILLUS SUBTILIS 34-61 PGIDB.sub.-- PSEPU GLUCOSE INHIBITED DIVISION PROTEIN B PSEUDOMONAS PUTIDA 25-52 PGLCP.sub.-- SYNY3 GLUCOSE TRANSPORT PROTEIN SYNECHOCYSTIS SP 288-322 PGLDA.sub.-- VACST GLYCEROL DEHYDROGENASE BACILLUS STEAROTHERMOPHILUS 20-79 PGLGA.sub.-- ECOLI GLYCOGEN SYNTHASE ESCHERICHIA COLI 256-283 PGLGC.sub.-- ECOLI GLUCOSE-1-PHOSPHATE ADENYLYLTRANSFERASE ESCHERICHIA COLI 114-141 PGLGC.sub.-- SALTY GLUCOSE-1-PHOSPHATE ADENYLYLTRANSFERASE SALMONELLA TYPHIMURIUM 114-141 PGLMS.sub.-- ECOLI GLUC-FRUC-6-PHOSAMINOTRANSFERASE ESCHERICHIA COLI 209-243 PGLN1.sub.-- METTL GLNB-LIKE PROTEIN 1 METHANOCOCCUS 58-85 THERMOLITHO TROPHICUS PGLNA.sub.-- ANASP GLUTAMINE SYNTHETASE ANABAENA SP 8-42 PGLNA.sub.-- BACSU GLUTAMINE SYNTHETASE BACILLUS SUBTILIS 4-31 PGLNA.sub.-- CLOAB GLUTAMINE SYNTHETASE CLOSTRIDIUM ACETOBUTYLICUM 413-440 PGLNA.sub.-- ECOLI GLUTAMINE SYNTHETASE ESCHERICHIA COLI 144-171 PGLNA.sub.-- METVO GLUTAMINE SYNTHETASE METHANOCOCCUS VOLTAE 203-230 PGLNA.sub.-- PROVU GLUTAMINE SYNTHETASE PROTEUS VULGARIS 142-169 PGLNA.sub.-- PYRFU GLUTAMINE SYNTHETASE PYROCOCCUS FURIOSUS 391-421 PGLNA.sub.-- SALTY GLUTAMINE SYNTHETASE SALMONELLA TYPHIMURIUM 144-171 PGLNA.sub.-- STRCO GLUTAMINE SYNTHETASE STREPTOMYCES COELICOLOR 186-213 PGLNB.sub.-- AZOBR NITROGEN REGULATORY PROTEIN P-II AZOSPIRILLUM BRASILENSE 15-49 PGLNB.sub.-- RHOCA NITROGEN REGULATORY PROTEIN P-II RHODOBACTER CAPSULATUS 15-49 PGLNB.sub.-- SYNP6 NITROGEN REGULATORY PROTEIN P-II SYNECHOCOCCUS SP 52-79

Detailed Description Paragraph Table Type 3 (48):
46-76 120-150 195-222 PIGGG.sub.-- STRSP IGG BINDING PROTEIN PRECURSOR STREPTOCOCCUS SP 46-76 120-150 195-225 270-297 PILVH.sub.-- ECOLI ACETOLACTATE SYNTHASE ESCHERICHIA COLI 47-81 120-147 PILVH.sub.-- SALTY ACETOLACTATE SYNTHASE SALMONELLA TYPHIMURIUM 47-81 120-147 PILVN.sub.-- LACLA ACETOLACTATE SYNTHASE LACTOCOCCUS LACTIS 20-75 PIMPB.sub.-- SALTY IMPB PROTEIN SALMONELLA TYPHIMURIUM 185-212 PIMP.sub.-- ACICA INOSINE-5'-MONOPHOSPHATE DEHYDROGENASE ACINETOBACTER CALCOACETICUS 166-193 PIMP.sub.-- BACSU E-5'-MONOPHOSPHATE DEHYDROGENASE BACILLUS SUBTILIS 159-186 PINA.sub.-- BACTL IMMUNE INHIBITOR A PRECURSOR BACILLUS THURINGIENSIS 103-130 324-358 PINLA.sub.-- LISMO INTERNALIN A LISTERIA MONCYTOGENES 106-143 161-188 196-232 PINLB.sub.-- LISMO INTERNALIN B PRECURSOR LISTERIA MONCYTOGENES 53-94 166-200 385-415 PINVA.sub.-- YEREN INVASIN YERSINIA ENTEROCOLITICA 501-535 PIPA7.sub.-- SHIFL 60 KD ANTIGEN SHIGELLA FLEXNERI 285-312 PIPAA.sub.-- SHIFL 70 KD ANTIGEN SHIGELLA FLEXNERI 95-136 437-475 493-557 596-630 PIPAB.sub.-- SHIDY 62 KD MEMBRANE ANTIGEN SHIGELLA DYSENTERIAE 28-55 71-169 480-507 522-556 PIPAB.sub.-- SHIFL 62 KD MEMBRANE ANTIGEN SHIGELLA FLEXNERI 28-55 71-169 480-507 522-556 PIPAC.sub.-- SHIDY 42 KD MEMBRANE ANTIGEN PRECURSOR SHIGELLA DYSENTERIAE 21-57 113-161 273-300 324-378 PIPAC.sub.-- SHIFL 42 KD MEMBRANE ANTIGEN PRECURSOR SHIGELLA FLEXNERI 28-57 113-161 273-300 324-372 PIPAD.sub.-- SHIDY 37 KD MEMBRANE ANTIGEN IPAD SHIGELLA DYSENTERIAE 47-86 291-318 PIPAD.sub.-- SHIFL 36 KD MEMBRANE ANTIGEN SHIGELLA FLEXNERI 47-86 259-286 291-318 PIPGB.sub.-- SHIDY IPGB PROTEIN SHIGELLA DYSENTERIAE 175-202 PIPGB.sub.-- SHIFL IPGB PROTEIN SHIGELLA FLEXNERI 175-202 PIPT.sub.-- PSESS ISOPENTENYL TRANSFERASE PSEUDOMONAS SYRINGAE 53-87 143-173 PIPYR.sub.--

ECOLI INORGANIC PYROPHOSPHATASE ESCHERICHIA COLI 138-172 PIRGA.sub.-- VIBCH VIRULENCE PROTEIN PRECURSOR VIBRIO CHOLERAE 212-239 336-377 PIRGB.sub.-- VIBCH VIRULENCE REGULATORY PROTEIN IRGB VIBRIO CHOLERAE 67-97 PIRPA.sub.-- SYNP7 IRON-REGULATED PROTEIN A SYNECHOCOCCUS SP 167-194 PISBD.sub.-- SHIDY INSERTION ELEMENT ISO-ISID PROTEIN INSB SHIGELLA DYSENTERIAE 86-113 PISBN.sub.- - SHIDY INSERTION ELEMENT ISO-ISID PROTEIN INSB SHIGELLA DYSENTERIAE 6-37 PISB.sub.-- ECOLI INSERTION ELEMENT IS1 PROTEIN INSB ESCHERICHIA COLI 122-149 PISB.sub.-- SHIFL INSERTION ELEMENT IS1 PROTEIN INSB SHIGELLA FLEXNERI 86-113 PISB.sub.-- SHISO INSERTION ELEMENT IS1 PROTEIN INSB SHIGELLA SONNEI 86-113 PISP1.sub.-- BACSU MAJOR INTRACELLULAR SERINE PROTEASE BACILLUS SUBTILIS 115-142 197-224 253-280 PSIP.sub.-- BACPO INTRACELLULAR SERINE PROTEASE BACILLUS POLYMYXA 109-143 PISTA.sub.-- ECOLI ISTA PROTEIN ESCHERICHIA COLI 183-210 PISTA.sub.-- SHISO ISTA PROTEIN SHIGELLA SONNEI 183-210 PIUTA.sub.-- ECOLI FERRIC AEROBACTIN RECEPTOR PRECURSOR ESCHERICHIA COLI 186-213 525-552 559-593 PJAG.sub.-- BACSU JAG PROTEIN BACILLUS SUBTILIS 68-95 PK6P2.sub.-- ECOLI 6-PHOSPHOFRUCTOKINASE ISOZYME 2 ESCHERICHIA COLI 143-170 PKAD.sub.-- BACSU ADENYLATE KINASE BACILLUS SUBTILIS 188-215 PKAD.sub.-- LACLA ADENYLATE KINASE LACTOCOCCUS LACTIS 186-213 PKANU.sub.-- BACSP KANAMYCIN NUCLEOTIDYLTRANSFERASE BACILLUS SP 69-96 PKANU.sub.-- STAAU KANAMYCIN NUCLEOTIDYLTRANSFERASE STAPHYLOCOCCUS AUREUS 69-96 PKDGT.sub.-- ECOLI 2-KETO-3-DEOXYGLUCONATE PERMEASE ESCHERICHIA COLI 70-97 PKDGT.sub.-- ERWCH 2-KETO-3-DEOXYGLUCONATE PERMEASE ERWINIA CHRYSANTHEMI 126-153 PKDTA.sub.-- ECOLI 3-DEOXY-D-MANNO-OCTULOSONIC-ACID TRANS ESCHERICHIA COLI 369-396 PKGTP.sub.-- ECOLI ALPHA-KETOGLUTARATE PERMEASE ESCHERICHIA COLI 7-34 PKGUA.sub.-- ECOLI GUANYLATE KINASE ESCHERICHIA COLI 162-189 PKHSE.sub.-- BACSU HOMOSERINE KINASE BACILLUS SUBTILIS 49-76 PKHSE.sub.-- FREDI HOMOSERINE KINASE FREMYELLA DIPLOSIPHON 52-79 PKKA4.sub.-- BACCI AMINOGLYCOSIDE 3'-PHOSPHOTRANSFERSE BACILLUS CIRCULANS 12-39 PKORB.sub.-- ECOLI KORB TRANSCRIPTIONAL REPRESSOR PROTEIN ESCHERICHIA COLI 228-255 PKPY1.sub.-- SPICI PYRUVATE KINASE SPIROPLASMA CITRI 112-148 PKPYK.sub.-- BACST PYRUVATE KINASE BACILLUS STEAROTHERMOPHILUS 331-374 PLACA.sub.-- STAAU ISOMERASE LACA SUBUNIT STAPHYLOCOCCUS AUREUS 9-64 PLACA.sub.-- STRMU ISOMERASE LACA SUBUNIT STREPTOCOCCUS MUTANS 26-60 PLACC.sub.-- STRMU TAGATOSE-6-PHOSPHATE KINASE STREPTOCOCCUS MUTANS 56-83 283-310 PLACG.sub.-- LACCA 6-PHOSPHO-BETA-GALACTOSIDASE LACTOBACILLUS CASEI 290-317 PLACI.sub.-- ECOLI LACTOSE OPERON REPRESSOR ESCHERICHIA COLI 9-36 PLACI.sub.-- KLEPN LACTOSE OPERON REPRESSOR KLEBSIELLA PNEUMONIAE 195-229 PLACR.sub.-- STAAU PHOSPHOTRANSFERASE REPRESSOR STAPHYLOCOCCUS AUREUS 2-29 PLACR.sub.-- STRMU PHOSPHOTRANSFERASE REPRESSOR STREPTOCOCCUS MUTANS 2-32 PLACY.sub.-- LACDE LACTOSE PERMEASE LACTOBACILLUS DELBRUECKII 196-230 PLAFB.sub.-- VIBPA PLAMB.sub.-- KLEPN MALTOPORIN PRECURSOR KLEBSIELLA PNEUMONIAE 337-364 PLAMI.sub.-- CLOTM ENDO-1,3(4)-BETA-GLUCANASE PRECURSOR CLOSTRIDIUM THERMOCELLUM 132-159 PLASI.sub.-- PSEAE OHHL SYNTHESIS PROTEIN LASI PSEUDOMONAS AERUGINOSA 171-198 PLCCI.sub.-- LEUGE PROBABLE LEUCOCIN A IMMUNITY PROTEIN LEUCONOSTOC GELIDUM 41-71 PLCNC.sub.-- LACLA LACTOCOCCIN A SECRETION PROTEIN LCNC LACTOCOCCUS LACTIS 162-189 207-234 388-433 PLCND.sub.-- LACLA LACTOCOCCIN A SECRETION PROTEIN LCND LACTOCOCCUS LACTIS 99-126 140-202 237-307 PLCRD.sub.-- YEREN LOW CALCIUM RESPONSE LOCUS PROTEIN D YERSINIA ENTEROCOLITICA 122-149 491-518 PLCRD.sub.-- YERPE LOW CALCIUM RESPONSE LOCUS PROTEIN D YERSINIA PESTIS 122-149 491-518 PLCRV.sub.-- YERPE VIRULENCE-ASSOCIATED V ANTIGEN YERSINIA PESTIS 22-49 157-184 240-267 PLCRV.sub.-- YERPS VIRULENCE-ASSOCIATED V ANTIGEN YERSINIA PSEUDOTUBERCULOSIS 22-49 240-267 PLCTB.sub.-- BACCA LCTB PROTEIN BACILLUS CALDOTENAX 18-45 PLCTB.sub.-- BACST LCTB PROTEIN BACILLUS STEAROTHERMOPHILUS 14-45 PLDHD.sub.-- LACPL D-LACTATE DEHYDROGENASE LACTOBACILLUS PLANTARUM 51-81 PHLHP.sub.-- BACPS D-LACTATE DEHYDROGENASE P BACILLUS PSYCHROSACCHAROLYTICUS 2-43 241-272 279-306 PLDHX.sub.-- BACPS D-LACTATE DEHYDROGENASE

X BACILLUS PSYCHROSACCHAROLYTICUS 2-43 241-275 279-306 PLDH.sub.-- BACME D-LACTATE DEHYDROGENASE BACILLUS MEGATERIUM 244-274 PLDH.sub.-- BACST D-LACTATE DEHYDROGENASE BACILLUS STEAROTHERMOPHILUS 241-268 279-313 PLDH.sub.-- BACSU D-LACTATE DEHYDROGENASE BACILLUS SUBTILIS 8-42 240-267 PLDH.sub.-- BIFLO D-LACTATE DEHYDROGENASE BIFIDOBACTERIUM LONGUM 22-49 PLDH.sub.-- LACPL D-LACTATE DEHYDROGENASE LACTOBACILLUS PLANTARUM 197-231 PLDH.sub.-- LISMO D-LACTATE DEHYDROGENASE LISTERIA MONOCYTOGENES 42-69 PLDH.sub.-- MYCHY D-LACTATE DEHYDROGENASE MYCOPLASMA HYOPNEUMONIAE 276-310 PLDH.sub.-- THEAQ D-LACTATE DEHYDROGENASE THERMUS AQUATICUS 3-30 PLEF.sub.-- BACAN LETHAL FACTOR PRECURSOR BACILLUS ANTHRACIS 165-192 304-331 480-514 548-578 619- 737- 658 764 PLEPA.sub.-- PSEFL LEPA PROTEIN PSEUDOMONAS FLUORESCENS 23-50 PLEP.sub.-- BACSU SIGNAL PEPTIDASE I BACILLUS SUBTILIS 3-30 PLEU1.sub.-- ECOLI 2-ISOPROPYLMALATE SYNTHASE ESCHERICHIA COLI 437-464 PLEU1.sub.-- LACLA 2-ISOPROPYLMALATE SYNTHASE LACTOCOCCUS LACTIS 22-49 379-484 PLEU3.sub.-- BACCO 3-ISOPROPYLMALATE DEHYDROGENASE BACILLUS COAGULANS 331-358 PLEU3.sub.-- CLOPA 3-ISOPROPYLMALATE DEHYDROGENASE CLOSTRIDIUM PASTEURIANUM 185-212 PLEUD.sub.-- LACLA 3-ISOPROPYLMALATE DEHYDRATASE LACTOCOCCUS LACTIS 163-190 PLEVR.sub.-- BACSU TRANSCRIPTIONAL REGULATORY PROTEIN LEVR BACILLUS SUBTILIS 297-324 676-703 744-774 785-822 PLEXA.sub.-- ERWCA LEXA REPRESSOR ERWINIA CAROTOVORA 146-173 PLIP1.sub.-- MORSP LIPASE 1 MORAXELLA SP 26-53 PLIP2.sub.-- MORSP LIPASE 2 MORAXELLA SP 356-383 PLIPB.sub.-- ECOLI LIPB PROTEIN ESCHERICHIA COLI 66-93 PLIP.sub.-- BURCE LIPASE PRECURSOR BURKHOLDERIA CEPACIA 176-203 PLIP.sub.-- PSEFL LIPASE PRECURSOR PSEUDOMONAS FLUORESCENS 8-35 PLIP.sub.-- PSES5 LIPASE PRECURSOR PSEUDOMONAS SP 176-203 PLIP.sub.-- STAAU LIPASE PRECURSOR STAPHYLOCOCCUS AUREUS 80-146 512-546 PLIVB.sub.-- SALTY LEU/ILE/VAL/THR-BINDING PROTEIN PRECURSOR SALMONELLA TYPHIMURIUM 193-220 PLIVC.sub.-- SALTY LEUCINE-SPECIFIC BINDING PROTEIN PRECURSOR SALMONELLA TYPHIMURIUM 195-222 PLIVE.sub.-- SALTY AMINO ACID TRANSPORT PROTEIN LIVE SALMONELLA TYPHIMURIUM 121-148 PLIVF.sub.-- ECOLI AMINO ACID TRANSPORT PROTEIN LIVF ESCHERICHIA COLI 23-50 PLIVJ.sub.-- CITFR LEU/ILE/VAL-BINDING PROTEIN PRECURSOR CITROBACTER FREUDII 195-222 PLIVJ.sub.-- ECOLI LEU/ILE/VAL-BINDING PROTEIN PRECURSOR ESCHERICHIA COLI 195-222 PLIVK.sub.-- ECOLI LEUCINE-SPECIFIC BINDING PROTEIN PRECURSOR ESCHERICHIA COLI 195-222 PLIVM.sub.-- ECOLI AMINO ACID TRANSPORT PROTEIN LIVM ESCHERICHIA COLI 121-148 PLKTA.sub.-- ACTAC LEUKOTOXIN ACTINOBACILLUS 113-147 173-213 398-443 451- 593- 655- ACTINOMYCETEMCOMITANS 488 620 711 PLKTA.sub.-- PASHA LEUKOTOXIN PASTEURELLA HAEMOLYTICA 53-99 179-216 345-372 409-436 455- 496- 545- 811- 853- 482 530 572 838 926 PLKTB.sub.-- ACTAC LEUKOTOXIN SECRETION PROTEIN ACTINOBACILLUS 487-514 ACTINOMYCETEMCOMITANS PLKTB.sub.-- PASHA LEUKOTOXIN SECRETION PROTEIN PASTEURELLA HAEMOLYTICA 42-69 78-105 488-515 PLKTC.sub.-- ACTAC LTC PROTEIN ACTINOBACILLUS 58-85 116-150 ACTINOMYCET EMCOMITANS PLKTC.sub.-- PASHA LKTC PROTEIN PASTEURELLA HAEMOLYTICA 123-157 PLKTD.sub.-- ACTAC LKTD PROTEIN ACTINOBACILLUS 116-164 205-242 278-305 364-391 ACTINOMYCETEMCOMITANS PLKTD.sub.-- PASHA LKTD PROTEIN PASTEURELLA HAEMOLYTICA 184-289 PLON.sub.-- ECOLI ATP-DEPENDENT PROTEASE LA ESCHERICHIA COLI 121-148 PLPXA.sub.-- RICRI UDP-N-ACETYLGLUCOSAMINE ACYLTRANSFERASE RICKETTSIA RICKETTSII 229-256 PLSPA.sub.-- ECOLI LIPOPROTEIN SIGNAL PEPTIDASE ESCHERICHIA COLI 10-37 PLSPA.sub.-- STAAU LIPOPROTEIN SIGNAL PEPTIDASE STAPHYLOCOCCUS AUREUS 134-161 PLUKF.sub.-- STAAU LEUKOCIDIN F SUBUNIT PRECURSOR STAPHYLOCOCCUS AUREUS 161-195 PLUKS.sub.-- STAAU LEUKOCIDIN S SUBUNIT PRECURSOR STAPHYLOCOCCUS AUREUS 157-207 PLUXA.sub.-- KRYAL ALKANAL MONOOXYGENASE ALPHA CHAIN KRYPTOPHANARON ALFREDI 190-217

Detailed Description Paragraph Table Type 3 (51):

PNOSZ.sub.-- PSEST NITROUS-OXIDE REDUCTASE PRECURSOR PSEUDOMONAS STUTZERI 557-591 PNPRE.sub.-- BACAM BACILLOLYSIN PRECURSOR BACILLUS AMYLOLIQUEFACIENS 113-147 217-244 PNPRE.sub.-- BACPO BACILLOLYSIN PRECURSOR BACILLUS POLYMYXA 57-91 187-228 PNPRE.sub.--

BACSU BACILLOLYSIN PRECURSOR BACILLUS SUBTILIS 116-146 307-334 PNQO5.sub.-- PARDE NADH-UBIQUINONE OXIDOREDUCTASE 21 KD CHAI PARACOCCUS DENITRIFICANS 4-45 PNQO9.sub.-- PARDE NADH-UBIQUINONE OXIDOREDUC 20 KD CHAIN PARACOCCUS DENITRIFICANS 125-152 PNRDD.sub.-- ECOLI ANAER RIBONUC-TRIPHOS REDUCTASE ESCHERICHIA COLI 91-125 PNRFA.sub.-- ECOLI CYTOCHROME C552 PRECURSOR ESCHERICHIA COLI 319-346 PNRFG.sub.-- ECOLI NRFG PROTEIN ESCHERICHIA COLI 72-111 PNRL1.sub.-- RHORH ALIPHATIC NITRILASE RHODOCOCCUS RHODOCHROUS 109-136 PNSR.sub.-- LACLA NISIN-RESISTANCE PROTEIN LACTOCOCCUS LACTIS 52-79 135-162 PNTCA.sub.-- ANASP DNA-BINDING PROTEIN VFI ANABAENA SP 65-92 PNTCA.sub.-- SYNP7 GLOBAL NITROGEN REGULATOR SYNECHOCOCCUS SP 44-91 PNTCA.sub.-- SYNY3 GLOBAL NITROGEN REGULATOR SYNECHOCYSTIS SP 67-94 PNTRB.sub.-- VIBAL NITROGEN REGULATION PROTEIN NTRB VIBRIO ALGINOLYTICUS 194-223 PNTRC.sub.-- PROVU NITROGEN REGULATION PROTEIN NR PROTEUS VULGARIS 385-412 PNTRC.sub.-- RHIME NITROGEN ASSIMILATION REGULATORY PROTEIN RHIZOBIUM MELILOTI 451-478 PNU2C.sub.-- SYNP7 NADH-PLASTOQUINONE OXIDOREDUCTASE CHAIN SYNECHOCOCCUS SP 80-107 PNU4C.sub.-- SYNY3 NADH-PLASTOQUINONE OXIDOREDUCTASE CHAIN SYNECHOCYSTIS SP 27-54 PNU5C.sub.-- SYNP2 NADH-PLASTOQUINONE OXIDOREDUCTASE CHAIN SYNECHOCOCCUS SP 614-641 PNUJC.sub.-- SYNY3 PROB NADH-UBIQUINONE OXIDOREDUCTASE SUB SYNECHOCYSTIS SP 163-190 PNUCK.sub.-- SYNY3 PROB NADH-UBIQUINONE OXIDOREDUCTASE SUB SYNECHOCYSTIS SP 169-199 PNULX.sub.-- SYNY3 NADH-PLASTOQUINONE OXIDOREDUCTASE SUB SYNECHOCYSTIS SP 46-90 PNUOG.sub.-- ECOLI NADH DEHYDROGENASE I CHAIN G ESCHERICHIA COLI 368-402 PNUOL.sub.-- ECOLI NADH DEHYDROGENASE I CHAIN L ESCHERICHIA COLI 30-57 496-523 PNUON.sub.-- ECOLI NADH DEHYDROGENASE I CHAIN N ESCHERICHIA COLI 392-419 PNUPC.sub.-- ECOLI NUCLEOTIDE PERMEASE ESCHERICHIA COLI 13-43 134-164 356-383 PNUSA.sub.-- ECOLI NUSA PROTEIN ESCHERICHIA COLI 21-62 PNUSB.sub.-- ECOLI N UTILIZATION SUBSTANCE PROTEIN B ESCHERICHIA COLI 17-65 70-97 PNUSG.sub.-- ECOLI TRANSCRIPTION ANTITERMINATION PROTEIN NUS ESCHERICHIA COLI 141-168 PNUSG.sub.-- THEMA TRANSCRIPTION ANTITERMINATION PROTEIN NUS THERMOTOGA MARITIMA 203-230 PNYLB.sub.-- FLASP 6-AMINOHEXANOATE-DIMER HYDROLASE FLAVOBACTERIUM SP 223-250 PNYLC.sub.-- FLASP 6-AMINOHEXANOATE-DIMER HYDROLASE FLAVOBACTERIUM SP 223-250 PO16G.sub.-- BACCE OLIGO-1,6-GLUCOSIDASE BACILLUS CEREUS 301-328 POCCT.sub.-- AGRT6 OCTOPINE-BINDING PROTEIN T PRECURSOR AGROBACTERIUM TUMEFACIENS 172-202 PODO1.sub.-- AZOVI 2-OXOGLUTARATE DEHYDROGENASE E1 COMPONE AZOTOBACTER VINELANDII 829-856 PODO1.sub.-- BACSU 2-OXOGLUTARATE DEHYDROGENASE E1 COMPONE BACILLUS SUBTILIS 487-524 809-850 PODO1.sub.-- ECOLI 2-OXOGLUTARATE DEHYDROGENASE E1 COMPONE ESCHERICHIA COLI 6-33 PODO2.sub.-- BACSU DIHYDROLIPOAMIDE SUC-TRANSF COMP BACILLUS SUBTILIS 30-60 PODOB.sub.-- PSEPU 2-OXOGLUTARATE DEHYDROGENASE E1 COMPONE PSEUDOMONAS PUTIDA 223-254 PODP1.sub.-- ECOLI PYRUVATE DEHYDROGENASE E1 COMPONENT ESCHERICHIA COLI 624-651 PODP2.sub.-- AZOVI DIHYDROLIPOAMIDE ACETRANS COMP AZOTOBACTER VINELANDII 518-545 PODP2.sub.-- ECOLI DIHYDROLIPOAMIDE ACETRANS COMP ESCHERICHIA COLI 14-41 117-144 PODPA.sub.-- BACST PYRUVATE DEHYDROGENASE E1 COMPONENT BACILLUS STEAROTHERMOPHILUS 299-333 PODPA.sub.-- BACSU PYRUVATE DEHYDROGENASE E1 COMPONENT BACILLUS SUBTILIS 305-332 PODPB.sub.-- BACST PYRUVATE DEHYDROGENASE E1 COMPONENT BACILLUS STEAROTHERMOPHILUS 23-50 PODPB.sub.-- BACSU PYRUVATE DEHYDROGENASE E1 COMPONENT BACILLUS SUBTILIS 16-50 POMIE.sub.-- CHLTR 15 KD CYSTEINE-RICH PROTEIN, SEROVAR E CHLAMYDIA TRACHOMATIS 38-65 POMA1.sub.-- NIEGO OUTER MEMBRANE PROTEIN PI.A PRECURSOR NEISSERIA GONORRHOEAE 63-90 POMA1.sub.-- NEIME OUTER MEMBRANE PROTEIN PI.A PRECURSOR NEISSERIA MENINGITIDIS 359-386 POMA2.sub.-- NEIME OUTER MEMBRANE PROTEIN PI.A PRECURSOR NEISSERIA MENINGITIDIS 353-380 POMB1.sub.-- NEIGO OUTER MEMBRANE PROTEIN P.IB PRECURSOR NEISSERIA GONORRHOEAE 63-90 POMB1.sub.-- NEIME OUTER MEMBRANE PROTEIN P.IB PRECURSOR NEISSERIA MENINGITIDIS 63-90 POMB2.sub.-- NEIGO OUTER MEMBRANE PROTEIN P.IB PRECURSOR

NEISSERIA GONORRHOEAE 63-90 POMB2.sub.-- NEIME OUTER MEMBRANE PROTEIN P.IB PRECURSOR NEISSERIA MENINGITIDIS 63-90 POMB3.sub.-- NEIME OUTER MEMBRANE PROTEIN P.IB PRECURSOR NEISSERIA MENINGITIDIS 63-90 POMB4.sub.-- NEIME OUTER MEMBRANE PROTEIN P.IB PRECURSOR NEISSERIA MENINGITIDIS 24-51 63-90 POMB.sub.-- NEILA OUTER MEMBRANE PROTEIN P.IB PRECURSOR NEISSERIA LACTAMICA 116-143 POMB.sub.-- NEISI OUTER MEMBRANE PROTEIN P.IB PRECURSOR NEISSERIA SICCA 24-51 63-90 POMLA.sub.-- ACTPL OUTER MEMBRANE LIPOPROTEIN PRECURSOR ACTINOBACILLUS PLEUROPNEUMONIAE 114-151 POMP1.sub.-- HAEIN OUTER MEMBRANE PROTEIN P1 PRECURSOR HAEMOPHILUS INFLUENZAE 154-184 303-330 341-368 POMP2.sub.-- HAEIN OUTER MEMBRANE PROTEIN P2 PRECURSOR HAEMOPHILUS INFLUENZAE 16-71 220-254 326-353 POMP3.sub.-- NEIGO OUTER MEMBRANE PROTEIN P.III PRECURSOR NEISSERIA GONORRHOEAE 14-41 POMP7.sub.-- STAAU 70 KD OUTER MEMBRANE PROTEIN PRECURSOR STAPHYLOCOCCUS AUREUS 53-80 88-115 POMPA.sub.-- THEMA OUTER MEMBRANE PROTEIN ALPHA PRECURSOR THERMOTOGA MARITIMA 100-138 151-178 183-249 255-292 301- 351- 328 385 POMPC.sub.-- ECOLI OUTER MEMBRANE PROTEIN C PRECURSOR ESCHERICHIA COLI 20-47 64-94 POMPC.sub.-- NEIGO OUTER MEMBRANE PROTEIN P.IIC PRECURSOR NEISSERIA GONORRHOEAE 89-123 POMPC.sub.- - SALTI OUTER MEMBRANE PROTEIN C PRECURSOR SALMONELLA TYPHI 166-193 POMPF.sub.-- ECOLI OUTER MEMBRANE PROTEIN F PRECURSOR ESCHERICHIA COLI 21-55 231-258 POMPH.sub.-- PHOS9 OMPH PROTEIN PHOTOBACTERIUM SP 292-319 POMPT.sub.-- ECOLI PROTEASE VII PRECURSOR ESCHERICHIA COLI 37-64 POP65.sub.-- NEIGO OPACITY PROTEIN OPA65 NEISSERIA GONORRHOEAE 71-111 POP67.sub.-- NEIGO OPACITY PROTEIN OPA67 NEISSERIA GONORRHOEAE 72-109 POPAA.sub.-- NEIGO OPACITY PROTEIN OPA53 NEISSERIA GONORRHOEAE 71-123 140-167 POPAG.sub.-- NEIGO OPACITY PROTEIN OPA52 NEISSERIA GONORRHOEAE 80-107 140-167 POPAI.sub.-- NEIGO OPACITY PROTEIN OPA54 NEISSERIA GONORRHOEAE 80-107 POPAJ.sub.-- NEIGO OPACITY PROTEIN OPA58 NEISSERIA GONORRHOEAE 71-105 POPKK.sub.-- NEIGO OPACITY PROTEIN OPA57 NEISSERIA GONORRHOEAE 71-105 POPDA.sub.-- ECOLI OLIGOPEPTIDASE A ESCHERICHIA COLI 147-174 POPDA.sub.-- SALTY OLIGOPEPTIDASE A SALMONELLA TYPHIMURIUM 147-174 POPDE.sub.-- PSEAE TRANSCRIPTION FACTOR OPDE PSEUDOMONAS AERUGINOSA 64-91 POPPA.sub.-- ECOLI OLIGOPEPTIDE-BINDING PROTEIN PRECURSOR ESCHERICHIA COLI 402-432 POPPB.sub.-- SALTY OLIGOPEPTIDE PERMEASE PROTEIN OPPB SALMONELLA TYPHIMURIUM 265-299 POPR1.sub.-- NEIME OPACITY-RELATED PROTEIN POPM1 NEISSERIA MENINGITIDIS 108-135 POPR3.sub.-- NEIME OPACITY-RELATED PROTEIN POPM3 NEISSERIA MENINGITIDIS 94-135 POSMC.sub.-- ECOLI OSMOTICALLY INDUCIBLE PROTEIN OSMC ESCHERICHIA COLI 5-31 POSPA.sub.-- BORBU OUTER SURFACE PROTEIN A PRECURSOR BORRELIA BURGDORFERI 63-100 112-139 157-204 223-271 POSPB.sub.- - BORBU OUTER SURFACE PROTEIN B PRECURSOR BORRELIA BURGDORFERI 113-259 262-296 POTC2.sub.-- BACSU ORNITHINE CARBAMOYLTRANSFERASE BACILLUS SUBTILIS 188-215 POTCC.sub.-- PSEAE ORNITHINE CARBAMOYLTRANSFERASE PSEUDOMONAS AERUGINOSA 17-44 POTCC.sub.-- PSEPU ORNITHINE CARBAMOYLTRANSFERASE PSEUDOMONAS PUTIDA 3-33 POUTB.sub.-- BACSU SPORE OUTGROWTH FACTOR B BACILLUS SUBTILIS 225-252 POUTO.sub.-- ERWCA LEADER PEPTIDASE ERWINIA CAROTOVORA 189-216 PP18K.sub.-- STRPA 18 KD PROTEIN IN FIMA 3'REGION STREPTOCOCCUS PARASANGUIS 115-149 PP18K.sub.-- STRSA 18 KD PROTEIN IN SSAB 3'REGION STREPTOCOCCUS SANGUIS 10-37 114-148 PP1P.sub.-- LACLC PI-TYPE PROTEINASE PRECURSOR LACTOCOCCUS LACTIS 107-155 904-950 1073- 1223- 1466- 1625- 1100 1250 1496 1655 PP29.sub.-- MYCHR PROTEIN P29 MYCOPLASMA HYORHINIS 5-56 101-160 202-246 PP2P.sub.-- LACLA PII-TYPE PROTEINASE PRECURSOR LACTOCOCCUS LACTIS 107-155 904-950 1073- 1223- 1466- 1625- 1100 1250 1496 1689 PP2P.sub.-- LACPS PII-TYPE PROTEINASE PRECURSOR LACTOBACILLUS PARACASEI 107-155 904-950 1073- 1223- 1466- 1628- 1100 1250 1496 1655 PP30.sub.-- ECOLI P30 PROTEIN ESCHERICHIA COLI 55-82 PP34.sub.-- RICRI PROTEIN P34 RICKETTSIA RICKETTSII 9-47 135-173 PP37.sub.-- MYCHR PROTEIN P37 PRECURSOR MYCOPLASMA HYORHINIS 38-75 PP3P.sub.-- LACLC PIII-TYPE PROTEINASE PRECURSOR LACTOCOCCUS LACTIS 107-155 904-950 1073- 1223- 1446- 1628- 1100 1250 1496 1655 PP47K.sub.-- PSECL 47 KD PROTEIN PSEUDOMONAS CHLORORAPHIS 288-315

PP54.sub.-- ENTFC P54 PROTEIN PRECURSOR ENTEROCOCCUS FAECIUM 58-92 141-209 PP60.sub.-- LISGR PROTEIN P60 PRECURSOR LISTERIA GRAYI 31-61 101-142 300-334 431-458 PP60.sub.-- LISIN PROTEIN P60 PRECURSOR LISTERIA INNOCUA 67-94 102-143 PP60.sub.-- LISIV PROTEIN P60 PRECURSOR LISTERIA IVANOVII 101-140 315-359 PP60.sub.-- LISMO PROTEIN P60 PRECURSOR LISTERIA MONOCYTOGENES 103-144 PP60.sub.-- LISSE PROTEIN P60 PRECURSOR LISTERIA SEELIGERI 101-140 270-298 321-365 395-422 PP60.sub.-- LISWE PROTEIN P60 PRECURSOR LISTERIA WELSHIMERI 113-140 317-361 396-423 PP69.sub.-- MYCHR PROTEIN P69 MYCOPLASMA HYORHINIS 264-295 421-464 487-517 544-575 PPABA.sub.-- BACSU ADC SYNTHASE BACILLUS SUBTILIS 12-41 PPABC.sub.-- BACSU 4-AMINO-4-DEOXYCHORISMATE LYASE BACILLUS SUBTILIS 250-277 PPABC.sub.-- ECOLI 4-AMINO-4-DEOXYCHORISMATE LYASE ESCHERICHIA COLI 140-167 PPABL.sub.-- STRGR PROTEIN Y STREPTOMYCES GRISEUS 52-79 PPAC.sub.-- ARTVI PENICILLIN ACYLASE PRECURSOR ARTHROBACTER VISCOSUS 170-197 333-363 571-606 640-674 PPAC.sub.-- BACSH PENICILLIN ACYLASE BACILLUS SPHAERICUS 232-259 PPAC.sub.-- STRMU PAC PROTEIN PRECURSOR STREPTOCOCCUS MUTANS 146-276 281-465 538-565 576-630 1075- 1159- 1381- 1102 1186 1434 PPAI1.sub.-- BACSU REGULATORY PROTEIN PAI 1 BACILLUS SUBTILIS 103-137 PPAI2.sub.-- BACSU REGULATORY PROTEIN PAI 2 BACILLUS SUBTILIS 145-172 PPAPE.sub.-- ECOLI FIMBRIAL PROTEIN PAPEESCHERICHIA COLI 42-69 86-123 PPAPF.sub.-- ECOLI MINOR FIMBRIAL PROTEIN PAPF ESCHERICHIA COLI 4-31 PPAPG.sub.-- ECOLI FIMBRIAL PROTEIN PAPG PRECURSOR ESCHERICHIA COLI 282-316 PPARA.sub.-- AGRTU PARA PROTEIN AGROBACTERIUM TUMEFACIENS 60-87 PPARB.sub.-- ECOLI PLASMID PARTITION PAR B PROTEIN ESCHERICHIA COLI 117-154 249-283 PPARE.sub.-- ECOLI TOPOISOMERASE IV SUBUNIT B ESCHERICHIA COLI 444-471 526-553 PPARE.sub.-- SALTY TOPOISOMERASE IV SUBUNIT B SALMONELLA TYPHIMURIUM 444-471 526-553 PPA.sub.-- BACAP PROTECTIVE ANTIGEN PRECURSOR BACILLUS ANTHRACIS 13-52 125-152 296-335 585-615 650- 684 PPBP2.sub.-- ECOLI PENICILLIN-BINDING PROTEIN 2 ESCHERICHIA COLI 95-122 178-205 207-241 PPBP2.sub.-- NEIGO PENICILLIN-BINDING PROTEIN 2 NEISSERIA GONORRHOEAE 193-220 PPBP2.sub.-- NEIME PENICILLIN-BINDING PROTEIN 2 NEISSERIA MENINGITIDIS 193-220

Detailed Description Paragraph Table Type 3 (54):
PRECQ.sub.-- ECOLI DNA HELICASE RECQ ESCHERICHIA COLI 468-495 PRELA.sub.-- ECOLI GTP PYROPHOSPHOKINASE ESCHERICHIA COLI 680-707 PREMA.sub.-- BACSU REPLICATION AND MAINTENANCE PROTEIN BACILLUS SUBTILIS 2-36 81-108 PREMA.sub.-- STAAU REPLICATION AND MAINTENANCE PROTEIN STAPHYLOCOCCUS AUREUS 2-36 81-108 PREMA.sub.-- STAEP REPLICATION AND MAINTENANCE PROTEIN STAPHYLOCOCCUS EPIDERMIDIS 2-36 81-108 PREP5.sub.-- ECOLI REPLICATION PROTEIN REPA ESCHERICHIA COLI 50-77 90-117 PREPA.sub.-- BACSU REPA PROTEIN BACILLUS SUBTILIS 342-373 PREPA.sub.-- ECOLI REPA PROTEIN ESCHERICHIA COLI 91-118 228-255 PREPA.sub.-- NEIGO REPLICATION PROTEIN NEISSERIA GONORRHOEAE 57-84 138-172 PREPB.sub.-- LACPL REPLICATION PROTEIN REPB LACTOBACILLUS PLANTARUM 184-211 PREPM.sub.-- STAAU REPLICATION INITIATION PROTEIN STAPHYLOCOCCUS AUREUS 254-284 PREPN.sub.-- STAAU REPLICATION INITIATION PROTEIN STAPHYLOCOCCUS AUREUS 258-285 PREPR.sub.-- STRAG REPR PROTEIN STREPTOCOCCUS AGALACTIAE 430-467 PREPS.sub.-- STRPY REPS PROTEIN STREPTOCOCCUS PYOGENES 423-467 PREPX.sub.-- STAAU REP PROTEIN STAPHYLOCOCCUS AUREUS 111-150 172-210 PREPY.sub.-- ECOLI REPLICATION INITIATION PROTEIN ESCHERICHIA COLI 288-315 PREP.sub.-- CLOPE REPLICATION PROTEIN CLOSTRIDIUM PERFRINGENS 168-195 297-324 343-375 PREP.sub.-- ECOLI REP HELICASE ESCHERICHIA COLI 119-146 205-243 PREP.sub.-- LACPL REP PROTEIN LACTOBACILLUS PLANTARUM 119-199 260-287 PRESP.sub.-- CLOPE RESOLVASE CLOSTRIDIUM PERFRINGENS 68-102 151-185 PRF2.sub.-- VACSU PROBABLE PEPTIDE CHAIN RELEASE FACTOR 2 BACILLUS SUBTILIS 34-68 PRF2.sub.-- ECOLI PEPTIDE CHAIN RELEASE FACTOR 2 ESCHERICHIA COLI 86-113 163-204 PRF2.sub.-- SALTY PEPTIDE CHAIN RELEASE FACTOR 2 SALMONELLA TYPHIMURIUM 86-113 163-204 PRF3.sub.-- ECOLI PEPTIDE CHAIN RELEASE FACTOR 3 ESCHERICHIA COLI 180-210 443-437 PRFAB.sub.-- ECOLI 1,6-GALACTOSYLTRANSFERASE ESCHERICHIA COLI 199-226 PRFAG.sub.--

ECOLI BIOSYNTHESIS PROTEIN RFAG ESCHERICHIA COLI 185-212 PRFAJ.sub.-- ECOLI
1,2-GLUCOSYLTRANSFERASE ESCHERICHIA COLI 39-66 233-268 PRFAJ.sub.-- SALTY
1,2-GLUCOSYLTRANSFERASE SALMONELLA TYPHIMURIUM 68-95 145-172 236-263 PRFAK.sub.-- SALTY
1,2-N-ACETYLGLUCOSAMINETRANSFERSE SALMONELLA TYPHIMURIUM 335-369 PRFAL.sub.-- ECOLI
O-ANTIGEN LIGASE ESCHERICHIA COLI 366-393 PRFAL.sub.-- SALTY O-ANTIGEN LIGASE
SALMONELLA TYPHIMURIUM 326-360 PRFAP.sub.-- ECOLI BIOSYNTHESIS PROTEIN RFAP
ESCHERICHIA COLI 8-35 PRFAS.sub.-- ECOLI BIOSYNTHESIS PROTEIN RFAS ESCHERICHIA COLI
62-89 184-240 PRFAY.sub.-- ECOLI BIOSYNTHESIS PROTEIN RFAY ESCHERICHIA COLI 18-45
PRFAZ.sub.-- ECOLI BIOSYNTHESIS PROTEIN RFAZ ESCHERICHIA COLI 111 3-30 85-112 PRFBB.sub.--
SALTY DTDP-GLUCOSE 4,6-DEHYDRATASE SALMONELLA TYPHIMURIUM 320-359 PRFBM.sub.-- SALTY
MANNOSE-1-PHOSPHATE GUANYLYLTRANSFERASE SALMONELLA TYPHIMURIUM 313-361 PRFBS.sub.--
SALTI PARATOSE SYNTHASE SALMONELLA TYPHI 22-56 205-232 PRFEA.sub.-- VIBAN PRECURSOR
FOR FERRIC ANGUIBACTIN VIBRIO ANGUILLARUM 349-376 PRFH.sub.-- ECOLI PEPTIDE CHAIN
RELEASE FACTOR HOMOLOG ESCHERICHIA COLI 83-110 PRGI2.sub.-- BACTU PUTATIVE GI2
SITE-SPECIFIC RECOMBINASE BACILLUS THURINGIENSIS 15-68 190-262 310-383 PRHAB.sub.-- ECOLI
RHAMNULOKINASE ESCHERICHIA COLI 175-202 PRHAB.sub.-- SALTY RHAMNULOKINASE
SALMONELLA TYPHIMURIUM 175-202 PRHAR.sub.-- ECOLI L-RHAMNOSE OPERON TRANSACTIVATOR
ESCHERICHIA COLI 10-41 PRHAS.sub.-- ECOLI L-RHAMNOSE OPERON REG PROTEIN RHAS
ESCHERICHIA COLI 152-179 PRHIR.sub.-- RHILV RHIR REGULATORY PROTEIN RHIZOBIUM
LEGUMINOSARUM 206-233 PRHLB.sub.-- ECOLI RNA HELICASE RHLB/MMRA ESCHERICHIA COLI
138-165 PRHO.sub.-- BORBU TRANS TERM FACTOR RHO BORRELIA BURGDORFERI 215-242 327-369
PRHPR.sub.-- BACSU PROTEASE PROD REG PROTEIN HPR BACILLUS SUBTILIS 82-109 PRHSA.sub.--
ECOLI RHSA PROTEIN PRECURSOR ESCHERICHIA COLI 667-694 PRHSB.sub.-- ECOLI RHSB PROTEIN
PRECURSOR ESCHERICHIA COLI 667-694 PRHSC.sub.-- ECOLI RHSC PROTEIN PRECURSOR
ESCHERICHIA COLI 380-414 667-694 1056- 1083 PRHSD.sub.-- ECOLI RHSD PROTEIN PRECURSOR
ESCHERICHIA COLI 671-712 1071- 1098 PRHSE.sub.-- ECOLI RHSE PROTEIN ESCHERICHIA COLI
345-372 PRIML.sub.-- ECOLI ACETYLTRANSFERASE ESCHERICHIA COLI 93-127 PRIR2.sub.-- ECOLI
RIBONUCLEOSIDE-DIPHOSPHATE REDUCTASE ESCHERICHIA COLI 167-194 PRISA.sub.-- PHOLE
RIBOFLAVIN SYNTHASE ALPHA CHAIN PHOTOBACTERIUM LEIOGNATHI 2-47 131-158 PRISB.sub. --
BACSU RIBOFLAVIN SYNTHASE BETA CHAIN BACILLUS SUBTILIS 8-35 PRISB.sub.-- PHOLE
RIBOFLAVIN SYNTHASE BETA CHAIN PHOTOBACTERIUM LEIOGNATHI 14-41 PRL10.sub.-- STRAT 50S
RIBOSOMAL PROTEIN L10 STREPTOMYCES ANTIBIOTICUS 14-72 106-133 PRL12.sub.-- SYNY3 50S
RIBOSOMAL PROTEIN L12 SYNECHOCYSTIS SP 2-34 PRL12.sub.-- THEMA 50S RIBOSOMAL PROTEIN
L12 THERMOTOGA MARITIMA 8-35 56-86 PRL14.sub.-- BACST 50S RIBOSOMAL PROTEIN L14 BACILLUS
STEAROTHERMOPHILUS 18-45 PRL14.sub.-- MICLU 50S RIBOSOMAL PROTEIN L14 MICROCOCCUS
LUTEUS 18-45 PRL14.sub.-- MYCCA 50S RIBOSOMAL PROTEIN L14 MYCOPLASMA CAPRICOLUM 51-92
PRL15.sub.-- BACLI 50S RIBOSOMAL PROTEIN L15 BACILLUS LICHENIFORMIS 21-48 PRL15.sub.--
BACST 50S RIBOSOMAL PROTEIN L15 BACILLUS STEAROTHERMOPHILUS 95-134 PRL15.sub.-- BACSU
50S RIBOSOMAL PROTEIN L15 BACILLUS SUBTILIS 95-122 PRL15.sub.-- CHLTR 50S RIBOSOMAL
PROTEIN L15 CHLAMYDIA TRACHOMATIS 110-144 PRL15.sub.-- ECOLI 50S RIBOSOMAL PROTEIN L15
ESCHERICHIA COLI 79-113 PRL15.sub.-- LACLA 50S RIBOSOMAL PROTEIN L15 LACTOCOCCUS LACTIS
8-35 PRL15.sub.-- METVA 50S RIBOSOMAL PROTEIN L15 METHANOCOCCUS VANNIELII 68-102
PRL15.sub.-- MYCCA 50S RIBOSOMAL PROTEIN L15 MYCOPLASMA CAPRICOLUM 63-135 PRL18.sub.--
BACST 50S RIBOSOMAL PROTEIN L18 BACILLUS STEAROTHERMOPHILUS 31-58 PRL18.sub.-- CHLTR
50S RIBOSOMAL PROTEIN L18 CHLAMYDIA TRACHOMATIS 32-86 PRL18.sub.-- HALMA 50S
RIBOSOMAL PROTEIN L18 HALOARCULA MARISMORTUI 80-107 PRL18.sub.-- MYCCA 50S RIBOSOMAL
PROTEIN L18 MYCOPLASMA CAPRICOLUM 61-88 PRL19.sub.-- ECOLI 50S RIBOSOMAL PROTEIN L19
ESCHERICHIA COLI 25-52 PRL19.sub.-- HALMA 50S RIBOSOMAL PROTEIN L19 HALOARCULA
MARISMORTUI 101-128 PRL19.sub.-- METVA PROBABLE 50S RIBOSOMAL PROTEIN METHANOCOCCUS
VANNIELII 45-72 PRL1.sub.-- PROVU 50S RIBOSOMAL PROTEIN L1 PROTEUS VULGARIS 159-194

PRL1.sub.-- SULSO 50S RIBOSOMAL PROTEIN L1 SULFOLOBUS SOLFATARICUS 5-32 184-211 PRL20.sub.-- ECOLI 50S RIBOSOMAL PROTEIN L20 ESCHERICHIA COLI 14-41 PRL20.sub.-- MYCFE 50S RIBOSOMAL PROTEIN L20 MYCOPLASMA FERMENTANS 14-41 PRL21.sub.-- BACSU 50S RIBOSOMAL PROTEIN L21 BACILLUS SUBTILIS 4-38 PRL22.sub.-- ECOLI 50S RIBOSOMAL PROTEIN L22 ESCHERICHIA COLI 28-55 PRL23.sub.-- METVA 50S RIBOSOMAL PROTEIN L23 METHANOCOCCUS VANNIELII 30-57 PRL23.sub.-- MYCCA 50S RIBOSOMAL PROTEIN L23 MYCOPLASMA CAPRICOLUM 32-59 PRL24.sub.-- HALMA 50S RIBOSOMAL PROTEIN L24 HALOARCULA MARISMORTUI 48-75 PRL24.sub.-- METVA 50S RIBOSOMAL PROTEIN L24 METHANOCOCCUS VANNIELII 61-90 PRL24.sub.-- MICLU 50S RIBOSOMAL PROTEIN L24 MICROCOCCUS LUTEUS 36-63 PRL29.sub.-- CHLTR 50S RIBOSOMAL PROTEIN L29 CHLAMYDIA TRACHOMATIS 39-66 PRL29.sub.-- ECOLI 50S RIBOSOMAL PROTEIN L29 ESCHERICHIA COLI 36-63 PRL29.sub.-- MYCCA 50S RIBOSOMAL PROTEIN L29 MYCOPLASMA CAPRICOLUM 39-85 PRL4.sub.-- BACST 50S RIBOSOMAL PROTEIN L4 BACILLUS STEAROTHERMOPHILUS 141-169 PRL4.sub.-- MYCCA 50S RIBOSOMAL PROTEIN L4 MYCOPLASMA CAPRICOLUM 144-198 PRL5.sub.-- THETH 50S RIBOSOMAL PROTEIN L5 THERMUS AQUATICUS 38-65 PRL6.sub.-- BACST 50S RIBOSOMAL PROTEIN L6 BACILLUS STEAROTHERMOPHILUS 79-106 PRL6.sub.-- ECOLI 50S RIBOSOMAL PROTEIN L6 ESCHERICHIA COLI 19-46 PRL6.sub.-- METVA 50S RIBOSOMAL PROTEIN L6 METHANOCOCCUS VANNIELII 129-159 PRL9.sub.-- BACST 50S RIBOSOMAL PROTEIN L9 BACILLUS STEAROTHERMOPHILUS 47-77 PRL9.sub.-- ECOLI 50S RIBOSOMAL PROTEIN L9 ESCHERICHIA COLI 122-149 PRLA0.sub.-- HALCU ACIDIC RIBOSOMAL PROTEIN P0 HOMOLOG HALOBACTERIUM CUTIRUBRUM 138-182 PRLA0.sub.-- HALHA ACIDIC RIBOSOMAL PROTEIN P0 HOMOLOG HALOBACTERIUM HALOBIUM 138-182 PRLA0.sub.-- HALMA ACIDIC RIBOSOMAL PROTEIN P0 HOMOLOG HALOARCULA MARISMORTUI 64-91 153-184 PRLA0.sub.-- METVA ACIDIC RIBOSOMAL PROTEIN P0 HOMOLOG METHANOCOCCUS VANNIELII 194-221 PRLA.sub.-- HALEU RIBOSOMAL PROTEIN `A` HALOPHILIC EUBACTERIUM NRCC 41227 59-86 PRLA.sub.-- HALHA 50S RIBOSOMAL PROTEIN L20 HALOBACTERIUM HALOBIUM 2-29 PRLA.sub.-- HALMA 50S RIBOSOMAL PROTEIN L12 HALOARCULA MARISMORTUI 2-29 PRLA.sub.-- METVA RIBOSOMAL PROTEIN `A` METHANOCOCCUS VANNIELII 2-29 PRLA.sub.-- MICLU 70S RIBOSOMAL PROTEIN MA MICROCOCCUS LUTEUS 55-82 90-117 PRLX1.sub.-- SALTY 43 KD RELAXATION PROTEIN SALMONELLA TYPHIMURIUM 226-260 PRLX1.sub.-- STAAU RLX PROTEIN STAPHYLOCOCCUS AUREUS 3-30 102-132 177-218 266-300 PRLX2.sub.-- SALTY 22 KD RELAXATION PROTEIN SALMONELLA TYPHIMURIUM 19-53 PRLX2.sub.-- STAAU RLX PROTEIN STAPHYLOCOCCUS AUREUS 3-30 102-133 261-295 PRLX3.sub.-- STAAU RLX PROTEIN STAPHYLOCOCCUS AUREUS 3-30 146-216 PRLX.sub.-- SULSO 50S RIBOSOMAL PROTEIN LX SULFOLOBUS SOLFATARICUS 32-62 PRNBR.sub.-- BACAM RIBONUCLEASE PRECURSOR BACILLUS AMYLOLIQUEFACIENS 33-67 129-156 PRNC.sub.-- ECOLI RIBONUCLEASE III ESCHERICHIA COLI 10-37 117-144 PRNE.sub.-- ECOLI RIBONUCLEASE E ESCHERICHIA COLI 413-440 628-662 PRNPA.sub.-- BUCAP RIBONUCLEASE P PROTEIN COMPONENT BUCHNERA APHIDICOLA 85-114 PRNPH.sub.-- BACSU RIBONUCLEASE PH BACILLUS SUBTILIS 159-186 PRNS.sub.-- ECOLI REGULATORY PROTEIN RNS ESCHERICHIA COLI 116-160 PRN.sub.-- VACCI RIBONUCLEASE BACILLUS CIRCULANS 82-109 PRN.sub.-- BACIN RIBONUCLEASE PRECURSOR BACILLUS INTERMEDIUS 38-72 PRP28.sub.-- BACTK RNA POLYMERASE SIGMA-28 FACTOR PRECURSOR BACILLUS THURINGIENSIS 73-107 PRP32.sub.-- CITFR RNA POLYMERASE SIGMA-32 FACTOR CITROBACTER FREUNDII 30-57 PRP35.sub.-- BACTK RNA POLYMERASE SIGMA-35 FACTOR PRECURSOR BACILLUS THURINGIENSIS 8-35 63-90 PRP54.sub.-- ALCEU RNA POLYMERASE SIGMA-54 FACTOR ALCALIGENES EUTROPHUS 229-266 PRP54.sub.-- AZOCA RNA POLYMERASE SIGMA-54 FACTOR AZORHIZOBIUM CAULINODANS 174-208 PRP54.sub.-- BACSU RNA POLYMERASE SIGMA-54 FACTOR BACILLUS SUBTILIS 16-43 97-124 274-308 396-423 PRP54.sub.-- BRAJA RNA POLYMERASE SIGMA-54 FACTOR 1 BRADYRHIZOBIUM JAPONICUM 97-124 PRP54.sub .-- KLEPN RNA POLYMERASE SIGMA-54 FACTOR KLEBSIELLA PNEUMONIAE 148-182 PRP54.sub.-- RHOCA RNA POLYMERASE SIGMA-54 FACTOR RHODOBACTER CAPSULATUS 155-185 PRP55.sub.-- BRAJA RNA POLYMERASE SIGMA-54 FACTOR 2 BRADYRHIZOBIUM JAPONICUM 145-172

☐ 8. Document ID: US 5853718 A

L1: Entry 8 of 16 File: USPT Dec 29, 1998

DOCUMENT-IDENTIFIER: US 5853718 A
TITLE: Method of immunization using biologically contained bacterial cells

Brief Summary Paragraph Right (68):
Examples of bacteria from which epitopes to be used in connection with the live vaccine of the invention may be derived, are enteric bacteria, e.g. pathogenic strains of Escherichia coli, Salmonella spp. such as S. typhimurium, S. typhi, S. schottmulleri and S. choleraesuis, Vibrio cholerae, Shigella dysenteriae; Corynebacterium diphteriae; Mycobacterium tuberculosis; Neisseria spp. such as N. gonorrhoeae, N. meningiditis and N. catarrhalis; Pseudomonas spp. such as P. aeruginosa; Yersinia spp. such as Y. pestis; Moraxella spp. such as M. bovis; Staphylococcus spp. such as S. aureus; Streptococcus spp. such as S. pneumoniae and S. pyogenes; Bordetella spp. such as B. pertussis and B. bronchiseptica; Hemophilus influenzae; Treponema pallidum; and Clostridium spp. such as C. botulinum and C. tetani.

Other Reference Publication (53):
Wu, et al.; Expression of Immunogenic Epitopes of Hepatitis B Surface Antigen With Hybrid Flagellin Proteins by a Vaccine Strain of Salmonella; PNAS (USA), 86:4726-30 (1989).

☐ 9. Document ID: US 5702916 A

L1: Entry 9 of 16 File: USPT Dec 30, 1997

DOCUMENT-IDENTIFIER: US 5702916 A
TITLE: Biological Containment

Brief Summary Paragraph Right (67):
Examples of bacteria from which epitopes to be used in connection with the live vaccine of the invention may be derived, are enteric bacteria, e.g. pathogenic strains of Escherichia coli, Salmonella spp. such as S.

typhimurium, S, typhi, S. schottmulleri and S. choleraesuis, Vibrio cholerae, Shigella dysenteriae; Corynebacterium diphteriae; Mycobacterium tuberculosis; Neisseria spp. such as N. gonorrhoeae, N. meningiditis and N. catarrhalis; Pseudomonas spp. such as P. aeruginosa; Yersinia spp. such as Y. pestis; Moraxella spp. such as M. bovis; Staphylococcus spp. such as S. aureus; Streptococcus spp. such as S. pneumoniae and S. pyogenes; Bordetella spp. such as B. pertussis and B. bronchiseptica; Hemophilus influenzae; Treponema pallidum; and Clostridium spp. such as C. botulinum and C. tetani.

Other Reference Publication (21):
Wu, et al.; Expression of Immunogenic Epitopes of Hepatitis B Surface Antigen with Hybrid Flagellin Protein by a Vaccine Strain of Salmonella; PNAS (USA), 86:4726-30 (1989).

☐ 10. Document ID: US 5670370 A

L1: Entry 10 of 16 File: USPT Sep 23, 1997

DOCUMENT-IDENTIFIER: US 5670370 A
TITLE: Biological containment

Brief Summary Paragraph Right (69):
Examples of bacteria from which epitopes to be used in connection with the live vaccine of the invention may be derived, are enteric bacteria, e.g. pathogenic strains of Escherichia coli, Salmonella spp. such as S. typhimurium, S. typhi, S. schottmulleri and S. choleraesuis, Vibrio cholerae, Shigella dysenteriae; Corynebacterium diphteriae; Mycobacterium tuberculosis; Neisseria spp. such as N. gonorrhoeae, N. meningiditis and N. catarrhalis; Pseudomonas spp. such as P. aeruginosa; Yersinia spp. such as Y. pestis; Moraxella spp. such as M. bovis; Staphylococcus spp. such as S. aureus; Streptococcus spp. such as S. pneumoniae and S. pyogenes; Borderella spp. such as B. pertussis and B. bronchiseptica; Hemophilus influenzae; Treponema pallidum; and Clostridium spp. such as C. botulinum and C. tetani.

Other Reference Publication (51):
Wu, et al.; Expression of Immunogenic Epitopes of Hepatitis B Surface Antigen with Hybrid Flagellin Proteins by a Vaccine Strain of Salmonella; PNAS (USA), 86:4726-30 (1989.

☐ 11. Document ID: US 5656488 A

DOCUMENT-IDENTIFIER: US 5656488 A
TITLE: Recombinant avirulent salmonella antifertility vaccines

Detailed Description Paragraph Right (51):
Recombinant plasmids containing one or more genes for the gamete-specific antigens can be introduced into one of several avirulent strains of bacteria containing mutations for genes necessary for long-term survival in the targeted host. Useful avirulent microbes include, but are not limited to, mutant derivatives of Salmonella and E. coli-Salmonella hybrids. Preferred microbes are members of the genus Salmonella such as S. typhimurium, S. typhi, S. paratyphi, S. gallinarum, S. pullorum, S. enteritidis, S. choleraesuis, S. arizona, or S. dublin. Avirulent derivatives of S. typhimurium and S. enteritidis find broad use among many hosts. Avirulent derivatives of S. gallinarum, S. pullorum and S. arizona may be particularly useful for immunizing avian species whereas S. typhimurium, S. typhi and S. paratyphi are preferred for use in humans. S. choleraesuis is preferably used to immunize swine while S. dublin finds use in cattle. The creation of such mutants is described in copending patent application Ser. No. 251,304 and in Curtiss and Kelly, 1987.

Detailed Description Paragraph Right (115):
It is evident that inclusion of the .DELTA.[crp-cysG]-10 or .DELTA.[crp-cysG]-14mutations which are also .DELTA.cdt mutations would enhance the safety of live attenuated Salmonella vaccine strains while not diminishing their immunogenicity. This might be particularly important for host-adapted invasive Salmonella species such as S. typhi, S. paratyphi A (S. schottmuelleri), S. paratyphi B (S. hirshfeldii), S. paratyphi C (all infect humans), S. choleraesuis (infects swine), S. dublin (infects cattle), S. gallinarum, and S. pullorum (both infect poultry), as well as non-host specific, invasive Salmonella species such as S. typhimurium and S. enteritidis.

Detailed Description Paragraph Right (134):
The pYA3054 construct can now be introduced into other .DELTA.cya .DELTA.crp .DELTA.asd vaccine strains, such as those derived from S. typhi described above, to impart human host specificity. Additional constructs producing LDH-C (human) at higher and lower levels, as periplasmic or surface components or fused to the B subunit of the labile toxin (LT) specified by enterotoxigenic E. coli, can be made as needed for comparisons.

Detailed Description Paragraph Right (165):
After optimizing the level of production of the LT-B-SP-10 fusion proteins in the recombinant avirulent Salmonella, the fusion proteins are purified. In order to avoid contaminating LPS, the .DELTA.crp .DELTA.asd S. typhimurium LT-2 derivative .chi.4153 which has a galE mutation to eliminate LPS core and O antigen production is used. Tn10 insertions in the genes for flagella and Type I pili are also introduced in the strain to avoid contaminating the antigen preparation with pilus and flagellar antigens. Cold osmotic shock is employed as the first step in the purification of the fusion protein. This generally gives a 15- to 20-fold purification over total cellular protein. Either a GM-1 affinity column (Tayot et al., 1981) or agarose (Clements and Finkelstein, 1979) is used, depending upon which gives the better affinity and reversibility of attachment to the fusion protein. The purity of the fusion protein is tested during development of the purification protocol measuring total proteins by standard methods and the fusion protein by quantitative ELISA. Purified fusion protein are lyophilized for long-term storage.

Detailed Description Paragraph Table (3):
pSD110.sup.+ .DELTA.[crp-cysG]-10 P22HTint(.lambda.3711) .chi.3945 with selection for

.DELTA.[zhc-1431::Tn10] .DELTA.cys-12 tetracycline resistance, Mal.sup.-, Cys.sup.-, zid-61::Tn10 Arg.sup.-. .chi.3957 pSD110.sup.+ .DELTA.[crp-cysG]-10 Fusaric acid-resistant, tetracycline- .DELTA.[zhc-1431::Tn10] .DELTA.cya-12 sensitive, Mal.sup.-, Cys.sup.-, Arg.sup.- derivative of .DELTA.[zid-61::Tn10] .chi.3956. .chi.3958 .DELTA.[crp-cysG]-10 .DELTA.[zhc-1431::Tn10] Ampicillin-sensitive derivative of .chi.3957; .DELTA.cya-12 .DELTA.[zid-61::Tn10] pSD110 cured by serial passage in L broth at 37.degree. C. .chi.3961 pSD110.sup.+ .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] P22HTint(.chi.3670) .chi.3954 with selection for ampicillin resistance, Mal.sup.+. .chi.3962 pSD110.sup.+ .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] P22HTint(.chi.3711) .chi.3961 with selection for .DELTA.cya-12 zid-62::Tn10 tetracycline resistance, Mal.sup.-. .chi.3978 pSD110.sup.+ .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] P22HTint(.chi.3711) .chi.3938 with selection for .DELTA.cya-12 zid-62::Tn10 tetracycline resistance, Mal.sup.-. .chi.3985 .DELTA.cya-12 .DELTA.[zid-62::Tn10] ATCC68166; Fusaric acid-resistant, .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] tetracycline-sensitive, Mal.sup.- derivative of .chi.3962 cured of pSD110. .chi.4038 .DELTA.cya-12 .DELTA.[zid-62::Tn10] Fusaric acid-resistant tetracycline- .DELTA.[crp-cysG]-10 .DELTA.[zhc-1431::Tn10] sensitive Mal.sup.-, Cys.sup.-, Arg.sup.- derivative of .chi.3902 cured of pSD110. .chi.4039 .DELTA.cya-12 .DELTA.[zid-62::Tn10] Fusaric acid-resistant, tetracycline- .DELTA.[crp-cysG]-10 .DELTA.[zhc-1431::Tn10] sensitive Mal.sup.- derivative of .chi.3978 cured of pSD110. .chi.4063 SR-11 arg::Tn10 P22HTint(Tn10 library) .chi.3306 with selection for tetracycline resistance, Arg.sup.-. .chi.4071 SR-11 arg::Tn10 P22HTint(Tn10 library) .chi.3306 with selection for tetracycline resistance, Arg.sup.-. .chi.4246 .DELTA.[crp-cysG]-10 zhc-1431::Tn10 P22HTint(.chi.3712) 798 with selection for tetracycline resistance, Mal.sup.-, (Cys.sup.- Arg.sup.-). .chi.4247 pSD110.sup.+ .DELTA.[crp-cysG]-10 P22HTint(.lambda.3670) .chi.4246 with selection for zhc-1431::Tn10 ampicillin resistance, Mal.sup.+, (Cys.sup.- Arg.sup.-). .chi.4248 .DELTA.[crp-cysG]-10 zhc-1431::Tn10 P22HTint(.lambda.3712) ATCC68169 (UK-1) with selection for tetracycline resistance, Mal.sup.-, (Cys.sup.- Arg.sup.-). .chi.4262 pSD110.sup.+ .DELTA.[crp-cysG]-10 P22HTint(.chi.3670) .chi.4248 with selection for zhc-1431::Tn10 ampicillin resistance, Mal.sup.+, (Cys.sup.- Arg.sup.-). C. S. typhi Ty2 Type E1 Cys.sup.- Trp.sup.- wild type Louis Baron, Walter Reed Army Institute of Research. ISP1820 Type 46 Cys.sup.- Trp.sup.- wild type Center for Vaccine Development, Baltimore, MD; 1983 isolate from Chilean patient. ISP2822 Type E1 Cys.sup.- Trp.sup.- wild type Center for Vaccine Development, Baltimore, MD; 1983 isolate from Chilean patient. .chi.3791 .DELTA.[crp-cysG]-10 zhc-1431::Tn10 P22HTint(.lambda.3712) ISP2822 with selection for tetracycline resistance (Mal.sup.-, Cys.sup.-, Arg.sup.-, Vi.sup.+). .chi.3792 .DELTA.[crp-cysG]-10 zhc-1431::Tn10 P22HTint(.lambda.3712) Ty2 with selection for tetracycline resistance (Mal.sup.-, Cys.sup.-, Arg.sup.- Vi.sup.+). .chi.3802 .DELTA.[crp-cysG]-10 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- sensitive Mal.sup.- derivative of .chi.3791 (Vi.sup.+). .chi.3803 .DELTA.[crp-cysG]-10 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- sensitive Mal.sup.- derivative of .chi.3792 (Vi.sup.+). .chi.3824 pSD110.sup.+ .DELTA.[crp-cysG]-10 .chi.3803 electro-transformed with pSD110 from .DELTA.[zhc-1431::Tn10] .chi.3670 with selection for ampicillin resistance (Mal.sup.+, Cys.sup.-, Arg.sup.-, Vi.sup.+). .chi.3845 pSD110.sup.+ .DELTA.[crp-cysG]-10 .chi.3802 electro-transformed with pSD110 from .DELTA.[zhc-1431::Tn10] .chi.3670 with selection for ampicillin resistance (Mal.sup.+, Cys.sup.-, Arg.sup.-, Vi.sup.+). .chi.3852 .DELTA.crp-11 zhc-1431::Tn10 P22HTint(.DELTA.3773) ISP2822 with selection for tetracycline resistance (Mal.sup.-, Vi+). .chi.3853 .DELTA.crp-11 zhc-1431::Tn10 P22HTint(.chi.3773) Ty2 with selection for tetracycline resistance (Mal.sup.-, Vi.sup.+). .chi.3877 .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- sensitive Mal.sup.- derivative of .chi.3852 (Vi.sup.+). .chi.3878 .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- sensitive Mal.sup.- derivative of .chi.3853 (Vi.sup.+). .chi.3879 pSD110.sup.+ .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] P22HTint(.chi.3670) .DELTA.3877 with selection for ampicillin resistance (Mal.sup.+, Vi.sup.+). .chi.3880 pSD110.sup.+ .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] P22HTint(.chi.3670) .chi.3878 with selection for ampicillin resistance (Mal.sup.+, Vi.sup.+). .chi.3919 pSD110.sup.+ .DELTA.[crp-cysG]-10 P22HTint(.chi.3711) .chi.3824 with selection for .DELTA.[zhc-1431::Tn10] .DELTA.cya-12 tetracycline resistance (Mal.sup.-, Vi.sup.+). zid-62::Tn10 .chi.3920 pSD110.sup.+ .DELTA.[crp-cysG]-10 P22HTint(.chi.3711) .chi.3845 with selection for .DELTA.[zhc-1431::Tn10] .DELTA.cya-12 tetracycline resistance (Mal.sup.-, Vi.sup.+). zid-62::Tn10 .chi.3921 pSD110.sup.+ .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] P22HTint(.chi.3711) .chi.3879 with selection

.DELTA.cya-12 zid-62::Tn10 for tetracycline resistance (Mal.sup.-, Vi.sup.+). .chi.3922 pSD110.sup.+ .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] P22HTint(.chi.3711) .chi.3880 with selection .DELTA.cya-12 zid-62::Tn10 for tetracycline resistance (Mal-, Vi.sup.+). .chi.3924 .DELTA.[crp-cysG]-10 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- .DELTA.cya-12 .DELTA.[zid-62::Tn10] sensitive Mal.sup.- derivative of .chi.3919 cured of pSD110 (Vi.sup.+). .chi.3925 .DELTA.[crp-cysG]-10 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- .DELTA.cya-12 .DELTA.[zid-62::Tn10] sensitive Mal.sup.- derivative of .chi.3920 cured of pSD110 (Vi.sup.+). .chi.3926 .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- .DELTA.cya-12 .DELTA.[zid-62::Tn10] sensitive Mal.sup.- derivative of .chi.3921 cured of pSD110 (Vi.sup.+). .chi.3927 .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- .DELTA.cya-12 .DELTA.[zid-62::Tn10] sensitive Mal.sup.- derivative of .chi.3922 cured of pSD110 (Vi.sup.+). .chi.3940 .DELTA.[crp-cysG]-10 .DELTA.[zhc-1431::Tn10] Flagella-positive, motile derivative of .DELTA.cya-12 .DELTA.[zid-62::Tn10] .chi.3925 (Vi.sup.+). .chi.4073 .DELTA.[crp-cysG]-10 .DELTA.[zhc-1431::Tn10] Flagella-positive, motile derivative of .DELTA.cya-12 .DELTA.[zid-62::Tn10] .chi.3924 (Vi.sup.+). .chi.4296 .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] P22HTint(.chi.3520) .chi.3927 with selection .DELTA.cya-12 .DELTA.[zid-62::Tn10] for tetracycline resistance and screening .DELTA.asdA1 zhf-4::Tn10 for Asd.sup.-, Mal.sup.-, Vi.sup.+. .chi.4297 .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- .DELTA.cya-12 .DELTA.[zid-62::Tn10] sensitive Asd.sup.-, Mal.sup.- derivative of .chi.4296 .DELTA.asdA1 .DELTA.[zhf-4::Tn10] (Vi.sup.+). .chi.4298 .DELTA.crp-11 zhc-1431::Tn10 P22HTint(.lambda.3773) ISP1820 with selection for tetracycline resistance (Mal.sup.-, Vi.sup.+). .chi.4299 .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] Fusaric acid-resistant, tetracycline- sensitive Mal.sup.- derivative of .chi.4298 (Vi.sup.+). .chi.4300 pSD110.sup.+ .DELTA.crp-11 .DELTA.[zhc-1431::Tn10] P22HTint(.chi.3670) .DELTA.4299 with selection for ampicillin resistance (Mal.sup.+, Vi.sup.+). .chi.4316

Detailed Description Paragraph Table (7):

TABLE 4

Virulence of S. typhimurium .DELTA.cya-12, .DELTA.crp-11, .DELTA.cya-12, and .DELTA.crp-11 Strains After Inoculation of BALB/c Mice with S. typhimurium .DELTA.cya-12 and/or .DELTA.crp-11 Strains Approx. Strain Relevant Route of Inoculating Survival wild-type Wild-type Number Genotype Inoculation Dose (CFU) live/Total Health.sup.a LD.sub.50 Origin

S. typhimurium .chi.3615 .DELTA.cya-12 PO 2 .times. 10.sup.9 5/5 healthy 6 .times. 10.sup.4 mouse .chi.3623 .DELTA.crp-11 PO 5 .times. 10.sup.8 5/5 healthy 6 .times. 10.sup.4 mouse .chi.3985 .DELTA.cya-12 .DELTA.crp-11 PO 2 .times. 10.sup.9 8/10 moderate 1 .times. 10.sup.5 horse .chi.4039 .DELTA.cya-12 .DELTA.crp-11 PO 1 .times. 10.sup.9 10/10 healthy 1 .times. 10.sup.5 pig S. typhi .chi.3926 .DELTA.cya-12 .DELTA.crp-11 IP.sup.b 2 .times. 10.sup.3 4/6 healthy .about.29 human .chi.3927 .DELTA.cya-12 .DELTA.crp-11 IP 3 .times. 10.sup.3 2/4 healthy <20 human

.sup.a Healthy-no noticeable signs of disease; moderatemoderately ill; illnoticeably ill. .sup.b IPcells delivered in 0.5 ml 5% hog gastric mucin.

☐ 12. Document ID: US 5643771 A

DOCUMENT-IDENTIFIER: US 5643771 A
TITLE: Non-reverting live bacterial vaccines

Brief Summary Paragraph Right (6):
Recently, bacterial strains have been developed for use as live vaccines which are streptomycin-dependent mutants of strains of several pathogenic species. Shigella flexneri and Shigella sonnei streptomycin-dependent mutants have been extensively used as live vaccines given by mouth for protection and have found to be both safe and efficient. In experimental Salmonella infections, however, streptomycin-dependent mutants seem to have been only moderately satisfactory. In general, "rough" mutants in Gram-negative bacterial species, i.e., mutants unable to manufacture normal lipopolysaccharide are non-virulent, but have proven unsatisfactory as live vaccines because of failure to cause protection. Two exceptions may be noted. (i) In Salmonella, mutation of gene galE prevents normal lipopolysaccharide synthesis unless the bacteria are provided with preformed galactose. A galE mutant of S. typhimurium was virtually non-virulent in small laboratory animals but evoked good immunity. As anti-O antibodies were produced, the galE bacteria must have obtained sufficient galactose within the host tissues for them to make at least some O-specific lipopolysaccharide. Recently a galE mutant of S. typhi, given by feeding to human volunteers, proved non-virulent and conferred reasonable protection against later oral challenge with a virulent strain of the same species. Furthermore, first reports of a field trial of this strain, given by oral route to school children in Alexandria, Egypt, indicate that it gave very good protection against the risk of contracting typhoid fever, which has a high incidence in such children. The non-virulence of galE strains seems to be conditional on the presence of normal host cellular defense mechanisms, since administration of the cytotoxic agent cyclophosphamide to mice previously injected with a galE mutant of S. typhimurium, non-pathogenic to untreated animals, precipitated fatal infections due to multiplication of the galE strain. (ii) A "rough" mutant of S. dublin is in routine use in Great Britain as a live vaccine, given by parenteral injection, for protection of newborn calves against the frequently fatal Salmonella infections which were formerly prevalent; as the strain used appears to lack the O-specific part of lipopolysaccharide, it presumably acts by invoking "non-specific immunity," perhaps by causing activation of macrophages.

Brief Summary Paragraph Right (8):
A non-virulent live vaccine may also serve as a host for the expression of antigens which may be located in the cytoplasm, translocated to the plasma or outer membrane or secreted to provide immunogens for an immunogenic reponse by the mammalian host. By employing a live vaccine as a carrier for an immunogen, particularly an invasive host, such as Salmonella typhi, a strong stimulus can be provided to the immune system, particularly to the humoral immune system. In this way, many of the benefits of employing attenuated live pathogens such as bacteria, fungi, protozoa and viruses can be achieved without concern for reversion to a virulent form.

Brief Summary Paragraph Right (14):
Hybrid vaccines comprising either Shigella modified by insertion of a DNA sequence from another organism or another organism modified by insertion of genes encoding Shigella antigens have been produced as a third approach. Formal et al., J. Bacteriol. (1965) 89:1374-1382 inserted a portion of the E. coli K12 chromosome into a virulent S. flexneri strain which produced an attenuated organism which failed to provide protection when used as a vaccine. Levine et al., J. Infect. Dis. (1977) 136:577-582 modified E. coli K12 by inserting the loci containing genes coding for the type-specific and group-specific factors of the O (LPS) antigen, which vaccine failed to provide protection. The addition of the invasive plasmid gene from S. flexneri to the

modified E. coli K12 described above produced a vaccine which appeared effective in tests in rhesus monkeys (Formal et al., Infect. Immun. (1984) 46:465-469). See also Formal et al., Infect Immun. (1981) 34:746-750 wherein S. sonnei O-antigen genes were introduced into S. typhi strain 21a.

Brief Summary Paragraph Right (25):
Among bacteria, the subject invention is particularly applicable to a wide variety of Salmonella strains, more particularly of groups A, B, or D, which includes most species which are specific pathogens of particular vertebrate hosts. Illustrative of the Salmonella causing disease for which live vaccines can be produced are S. typhimurium; S. typhi; S. abortus-ovi; S. abortus-equi; S. dublin; S. gallinarum; S. pullorum; as well as others which are known or may be discovered to cause infections in mammals.

Brief Summary Paragraph Right (38):
In accordance with the subject invention, the vaccines are produced by introducing a non-reverting mutation in at least one gene, where each mutation is of a sufficient number of bases in tandem to insure a substantially zero probability of reversion and assurance of the non-expression of each mutated gene, in the sense of its total inability to determine production of an enzymically active protein. In addition, each gene chosen will be involved in at least one, and preferably at least two, biosynthetic pathways to produce metabolites which are either infrequently present in the host or completely absent. The type of gene and number chosen will result in the likelihood that a host for the vaccine will provide the necessary nutrients for proliferation will have a probability approximating zero. These requirements have been shown to be fulfilled by the aroA and purA genes of Salmonella, particularly typhimurium dublin and typhi, so that these genes are preferred, although other genes, as previously indicated, may also serve as the site for the non-reverting mutation.

Detailed Description Paragraph Right (5):
The isolation of tetracycline-sensitive variants is facilitated by the fact that tetracycline, at appropriate concentrations, prevents multiplication of tetracycline-sensitive bacteria, but does not kill them, whereas penicillin kills multiplying bacteria but spares non-multiplying bacteria. The technique of penicillin selection was used for isolation of tetracycline-sensitive variants from the aroA::Tn10 strain SL3218. The strain was first grown in broth without tetracycline to a concentration of approximately 9.times.10.sup.8 cfu/ml; this culture was then diluted 1:10 into broth containing tetracycline, 5 .mu.g/ml, and the diluted culture incubated at 37.degree. with aeration for 75 min.; ampicillin, 2.5 .mu.g/ml, was added and incubation with shaking was continued for 290 min.; the culture was then held at room temperature without shaking overnight. Surviving bacteria were washed on a membrane filter to remove ampicillin, and then plated on an indicator medium containing dyes and a very low concentration of tetracycline. On this medium, tetracycline-sensitive bacteria produce small, dark colonies, whereas tetracycline-resistant bacteria produce large pale colonies. The ampicillin treatment reduced the number of viable bacteria by about 10.sup.-5. Six of 386 survivor colonies tested proved to be tetracycline-sensitive. Two such isolants, designated SL3235 and SL3236 were shown to resemble their parent strain SL3218 in nutritional character, but to differ from it not only by their tetracycline-sensitivity, but also by their failure to produce any aromatic independent revertants in tests which would have detected reversion at a frequency of one in 10.sup.11 /bacterium/generation. One of these strains, SL3235, when used as live vaccine in mice and calves, showed no reversion to aromatic independence of virulence. Another non-reverting aro.sup.- vaccine strain was prepared from S2357/65, a Salmonella typhimurium strain known from experiments elsewhere to be highly virulent for calves. Strain S2357/65 is prototrophic: to provide a marker character it was made by transduction first hisD8557::Tn10 (therefore phenotypically with a histidine requirement not satisfied by histidinol, and tetracycline-resistant), then by a second transduction made hisD.sup.+ hisG46 (thus phenotypically with requirement for histidine or histidinol, and tetracycline-sensitive). This derivative, SL323, was shown to cause fatal infection when fed to a calf. The calf-passage strain, labeled SL1344, was next made aroA544::Tn10 by transduction from TT1455, as described above; the hisG46 aroA544::Tn10 strain so obtained was labeled SL1346. A tetracycline-sensitive mutant, still aromatic-requiring but now unable to revert to aromatic independence, was next isolated from

SL1346 by a new method, i.e., selection on nutrient agar containing chlortetracycline, 50 .mu.g/ml, added before autoclaving, and fusaric acid, 12 .mu.g/ml, added after autoclaving. This medium prevents or at least greatly retards growth of tetracycline-resistant bacteria but allows good growth of tetracycline-sensitive bacteria. Both this strain and two aroA::Tn10 strains, SL3217 and SL3218 grown with tetracycline, to prevent accumulation of tetracycline-sensitive aro.sup.+, therefore virulent, revertants have been shown to be effective as live vaccine when administered to mice by intraperitoneal route. (1) Experiments with strains SL3217 and SL3218: CV1 mice given ca. 2.times.105 live vaccine-strain bacteria, i.p.: challenge two months later with 2.times.10.sup.6 bacteria (i.e. more than 20,000 LD50) of virulent S. typhimurium strain SL3201, i.p.: no deaths in two months' observation. (ii) CBA/N.times.DBA/LN F.sub.1 female mice given 10.sup.6 or 10.sup.5 live-vaccine strain SL3235, i.p.: challenged five weeks later with 10.sup.6 bacteria (ca. 100 LD50) of virulent S. typhimurium strain TML, o.p.: no deaths in fifteen days' observation. In other experiments, the stable aro.sup.- vaccine strain, SL3235, has been shown not to cause death (nor any obvious ill effects) when injected intraperitoneally even into mice exceptionally susceptible to Salmonella typhimurium infection, either in consequence of prior intravenous injection of macroparticulate silica, so as to estroy phagocytic function of macrophages, or in mice exceptionally susceptible because of a genetic defect in ability to respond to lipopolysaccharide, i.e., strain C3H/Heg. A non-reverting aromatic-dependent derivative thus obtained, number SL3261, has been shown to be non-virulent for mice and calves, by parenteral or oral routes. In addition, a derivative of type aroA::Tn10, similarly derived by transduction from a calf-virulent strain of the bovine-pathogenic species, Salmonella dublin, has been shown to be non-virulent for mice; and aroA::Tn10 derivatives, presumably non-virulent, have been made by transduction from several recently isolated strains of the human pathogen, Salmonella typhi.

Detailed Description Paragraph Right (20):
Tetracycline-resistant transductants were selected on "Oxoid" blood agar base, code CM55, supplemented with 25 .mu.g/ml tetracycline. For recipient strains deficient in aromatic biosynthesis, this medium was supplemented with 2,3-dihydroxybenzoic acid (DBA), to allow synthesis of enterobactin which is required for capture of ferric iron. For selection of transductants of altered nutritional character, a simple defined medium supplemented with tryptophan and cystine (requirements of wild-type S. typhi) was used.

Detailed Description Paragraph Right (21):
The transduction plates were inspected after 1, 2, 3 and 4 days of incubation, and colonies appearing in the drop areas were picked and purified by streaking out and selection of discrete colonies on the same medium as that on which selection was made. In general, transductant colonies developed later, and in much smaller numbers, e.g., by a factor of 10.sup.3 in crosses in which the recipient was S. typhi, as compared with those in which it was S. typhimurium, like the donor. This presumably resulted from incomplete genetic homology of the genes of the S. typhimurium donor with the corresponding genes of the S. typhi recipient, greatly reducing the frequency of crossing-over events and so of integration of donor genes into recipient chromosome. Purified transductant clones were tested to insure that they were S. typhi, not aerial contaminants, and to confirm that they were of the phenotype being sought.

Detailed Description Paragraph Right (26):
The first donor strain used was strain SL5173, which is S. typhimurium hisD8557::Tn10 having Tn10 inserted in gene hisD and causing inability to effect the last step in histidine biosynthesis and a requirement for histidine not satisfied by provision of histidinol. Lysate G2023 from phage grown on strain SL5173 was applied to S. typhi strain 515Ty having the aroA deletion as described above. Tetracycline-resistant transductants were selected, and after purification, tested for nutritional character. A clone with a histidine requirement not satisfied by provision of histidinol was selected and designated 521Ty.

Detailed Description Paragraph Right (31):
To introduce the purA155 deletion into 523Ty, a phage lysate of strain SL5475 was applied to the

tetracycline-sensitive S. typhi recipient strains and selection made for tetracycline-resistant transductants by the same procedure as described above for introduction of the aroA(serC)::Tn10 mutation. After single-colony reisolation, tetracycline-resistant transductant clones were tested for adenine requirement (in addition to their previous requirements for aromatic metabolites and histidine). A purA155 deletion zjb-906::Tn10 transductant was obtained and designated 531Ty.

Detailed Description Paragraph Right (33):
Tetracycline-sensitive mutants of 531Ty were obtained by spreading a diluted broth culture on a medium which hinders the growth of strains which are tetracycline-resistant because of presence of Tn10 (Bochner et al., J. Bacteriol. (1980) 143:926). This medium was modified by addition of 2,3-dihydrobenzoic acid, at about 1 .mu.g/ml, because of the aro defect of the S. typhi strain in use. The tetracycline-sensitive mutants thus obtained, resulting from deletion of the part of the transposon causing tetracycline-resistance, were checked to confirm that they were of unaltered nutritional character and that they had the antigenic characters of their S. typhi wild-type ancestor. One such isolate, designated 541Ty, constitutes a Vi-positive aro(deln.) his purA(deln.) tetracycline-sensitive live-vaccine strain in the CDC10-80 line.

Detailed Description Paragraph Right (37):
Clements et al., Infect. Immun. (1984) 46:564-569, report the preparation of the plasmid pJC217 containing the gene for expression of the 56 kD B region of the heat-labile enterotoxin operon of E. coli. The plasmid pJC217 is transformed into the strains 541Ty and 543Ty as described in Clements et al., supra. The expression of LT-B is confirmed by an ELISA assay. Microtiter plates are precoated with 7.5 .mu.g/well of mixed gangliosides (Type III), then with 1 .mu.g per well of purified LT-B. Reagents and antisera are available from Sgima Chemical Co. The samples are serially diluted in PBS(pH 7.2)-0.05% Tween-20. Ampicillin resistant colonies are tested for LT-B. The LT-B positive transformants are then used for immunization for both S. typhi and heat-labile enterotoxin as described previously.

Detailed Description Paragraph Right (75):
Salmonella typhimurium strain SL1479 was deposited at the ATCC on Sep. 7, 1982 and given ATCC Accession No. 39183; Salmonella dublin strain SL1438 was deposited on Sep. 7, 1982 at the ATCC and given ATCC Accession No. 39184. Salmonella typhi strain 531Ty was deposited at the ATCC on Nov. 21, 1984, and granted ATCC Accession No. 39926. Shigella flexneri strain SFL114 was deposited at the ATCC on Mar. 14, 1988 and given ATCC Accession No. 53755.

Other Reference Publication (6):
Stocker "Aromatic-Dependent Salmonella as Live Vaccine Presenters of Foriegn Epitopes as Inserts in Flagellin" Res. Microbiol 141:787 (1990).

Other Reference Publication (22):
Stocker, B.A.D., "Aromatic-Dependent Salmonella as Live Vaccine Presenters of Foreign Epitopes as Inserts in Flagellin", Res. Microbiol., 141:787 (1990).

Other Reference Publication (28):
Newton, S.M., et al., "Expression and Immunogenicity of a Streptococcal M Protein Epitope Inserted in Salmonella Flagellin", Infection and Immunity, 59:2158 (1991).

DOCUMENT-IDENTIFIER: US 5585102 A
TITLE: Flagella-less borrelia

Brief Summary Paragraph Right (15):
In another embodiment, the immunoassay may comprise what is known to those of skill in the art as a competitive immunoassay; in such an assay binding is detected by adding a preparation of labeled antibodies reactive with a selected flagella-less Borrelia strain to the contacted sample and measuring binding of the labeled antibody to the antigenic preparation. Because antibodies in the sample will compete with the labeled antibodies for antigenic epitopes in the antigen preparation, binding of the labeled antibody will be inversely proportional to the concentration of antibody in the sample.

Drawing Description Paragraph Right (1):
FIG. 1: The oligonucleotide 5'-*GCCAGCAGCATCATCAGAAG*-3' which represented a conserved sequence of fla genes of two other strains of B. burgdorferi (Gassmann, et al., 1989), was synthesized and used to identify a flagellin gene-bearing clone in a library of genomic DNA of strain HB19 of B. burgdorferi in .lambda.FIX II. A 5.0 kb Bgl II fragment containing the complete fla gene of strain HB19 was subcloned into the plasmid vector pBR322 to yield recombinant plasmid pACA1. The nucleotide sequence of both strands of the flagellin gene and its 5' and 3' flanking sequences in pACA1 were determined by primer-directed sequencing of double-stranded pACA1 plasmid DNA. The start of transcription of flagellin gene was identified by primer extension analysis of total RNA isolated from strain HB19 B. burgdorferi. The analysis revealed the following: (i) the coding region for the flagellin gene of strain HB19 from positions 58-1065; (ii) the transcriptional start site, the *C* at position +1, 57 bp distant from the start codon; (iii) the likely ribosomal binding site (RBS) as *GGAGG* at position 45 to 49; and (iv) the likely "-10" (*GCTATT*) and "-35" (*CGTT*) promoter boxes. The numbers in the top column refer to nucleotides, those in the bottom column to amino acids.

Detailed Description Paragraph Right (13):
In some circumstances, it may be desirable to mutagenize the starting population of B. burgdorferi, for example, with chemicals, such as nitrosoguanidine, or irradiation, such as gamma-rays, in order to increase the frequency of mutation. The mutagenized B. burgdorferi are then selected by cloning by limiting dilution or by colony formation as described above. Another procedure which could be used comprises transposon mutagenesis of the flagellin gene followed by selection in antibiotic-containing BSK medium without Yeastolate (BSK I), and further selection for flagella-less mutants as described above. With the aid of the present disclosure, one may also devise methods for preparing flagella-less strains by recombinant DNA technology, for example, in vitro mutagenesis of the cloned flagellin gene and transformation of the mutant gene back into the borrelia. The sequence of the cloned flagellin gene and the 3' and 5' flanking sequences is shown in FIG. 1. The cloned flagellin gene could be accompanied by an antibiotic selection marker to aid selection of transformants in broth medium or on solid medium. Antibiotic-resistant transformants would be examined as to flagella phenotype. With any of these procedures, the mutations may be in the gene itself or in the regulatory regions, such as the promoter or terminator, for the flagellin gene. More specifically, such mutations can include deletion of the entire coding region of the gene or portions thereof, deletion or mutagenesis of the ribosomal binding sequence (RBS), deletion or mutagenesis of the -10 and -35 promoter boxes, or insertion or deletion of DNA transcribed sequence of the gene such that a functional flagellar

protein is not produced.

Detailed Description Paragraph Type 1 (5):
5. A. G. Barbour et al., A Borrelia-specific monoclonal antibody binds to a flagellar epitope, Infect. Immun. 1986; 52:549-54.

Detailed Description Paragraph Type 1 (48):
48. F. Sadallah et al., Production of specific monoclonal antibodies to Salmonella typhi flagellin and possible application to immunodiagnosis of typhoid fever, J. Inf. Dis. 1990; 161:59-64.

Other Reference Publication (13):
Barbour, et al., "A Borrelia-Specific Monoclonal Antibody Binds to a Flagellar Epitope," Infect. Immun., 52:549-555 (1986).

☐ 14. Document ID: US 5554372 A

L1: Entry 14 of 16 File: USPT Sep 10, 1996

DOCUMENT-IDENTIFIER: US 5554372 A
TITLE: Methods and vaccines comprising surface-active copolymers

Detailed Description Paragraph Right (47):
Salmonella typhi organisms of strain TY2 are grown in motility agar. The highly motile organisms should be selected because they produced the most flagella. Organisms are then inoculated in 20 liters of trypticase soy broth and incubated at 37.degree. C. for approximately 30 hours until the end of the log phase of growth. The organisms may be killed at this time by the addition of formaldehyde to produce a 0.3% suspension. The organisms are preferably collected by centrifugation; however, care should be taken to avoid production of excessive shear force. The flagella are then removed from the organisms by shaking vigorously for 20 minutes in a shaker. Other mixes and devices which produce a shear force to break off the flagella without disrupting the organism are equally satisfactory.

Detailed Description Paragraph Right (54):
As an example, injections of 20 .mu.g of Salmonella typhi flagella conjugated with dinitrophenol resulted in IgG antibody titers specific for the hapten DNP which rose at the end of the first week after injection and persisted for over one year.

Detailed Description Paragraph Right (82):
Salmonella typhi organisms of strain TY2 are grown in motility agar. Organisms are then inoculated in 20 liters of trypticase soy broth and incubated at 37.degree. for 30 hours until the end of the log phase of growth. The organisms are killed at this time by the addition of formaldehyde to produce a 0.3% suspension.

The organisms are collected by centrifugation. Care should be taken to avoid production of excessive shear force. The flagella are then removed from the organisms by shaking vigorously for 20 minutes in a shaker. Other mixes and devices which produce a shear force to break off the flagella without disrupting the organism are equally satisfactory.

Detailed Description Paragraph Right (141):
Salmonella typhi, strain TY2 (type 29), is obtained from the American Type Culture Collection. Frozen stock cultures are grown on Tryptic Soy Agar plates (Difco Laboratories, Detroit, Mich.) and passaged 4-5 times through 0.3% Tryptic Soy Motility Agar. The highly motile bacteria are selected because they produce the most flagella. Organisms are inoculated into Tryptic Soy Broth and incubated at 37.degree. C. for 6 hours. Aliquots of the broth suspension of bacteria are inoculated onto Mueller Hinton Agar plates (Carr Scarlborough) incubated at 37.degree. C. for 16 hours. The cells are harvested off the plates with PBS containing 0.1% thimerosal (Sigma Chemical Co., St Louis, Mo.). The flagella are removed from the cells by vigorous shaking for 20 minutes in a mechanical shaker (Red Devil Paint Shaker) and separated from the cell bodies by differential centrifugation as follows: the cell bodies are pelleted by centrifugation at 6000.times.9 for 30 minutes in a Sorvall RC-5B refrigerated Superspeed Centrifuge (DuPont Instruments) with a GSA rotor, followed by centrifugation at 16,000.times.9 for 10 minutes to pellet broken cells and other small debris. Flagella are then pelleted at 90,000.times.9 in a Beckman L8-70M ultracentrifuge with a SW27 swing bucket rotor, resuspended in thimerosal-PBS, repelleted, and resuspended in thimerosal-PBS. Protein concentration is determined by Lowry's Protein Determination..sup.31 Aliquots of 5.2 mg/ml flagella are frozen at -70.degree. C.

Detailed Description Paragraph Type 0 (32):
Salmonella species, Salmonella typhi

Other Reference Publication (6):
Majarian et al., "Expression of heterologous epitopes as recombinant flagella on the surface of attenuated Salmonella", Vaccines 89, pp. 277-281 (1989).

Other Reference Publication (7):
Netwon et al., "Immune response to cholera toxin epitope inserted in Salmonella flagellin", Science, vol. 244, pp. 70-72 (1989).

☐ 15. Document ID: US 5436000 A

L1: Entry 15 of 16 File: USPT Jul 25, 1995

DOCUMENT-IDENTIFIER: US 5436000 A
TITLE: Flagella-less borrelia

Brief Summary Paragraph Right (15):
In another embodiment, the immunoassay may comprise what is known to those of skill in the art as a competitive immunoassay; in such an assay binding is detected by adding a preparation of labeled antibodies reactive with a selected flagella-less Borrelia strain to the contacted sample and measuring binding of the labeled antibody to the antigenic preparation. Because antibodies in the sample will compete with the labeled antibodies for antigenic epitopes in the antigen preparation, binding of the labeled antibody will be inversely proportional to the concentration of antibody in the sample.

Drawing Description Paragraph Right (1):
FIG. 1: The oligonucleotide 5'-GCCAGCAGCATCATCAGAAG-3' (SEC ID No: 3) which represented a conserved sequence of fla genes of two other strains of B. burgdorferi (Gassmann, et al., 1989), was synthesized and used to identify a flagellin gene-bearing clone in a library of genomic DNA of strain HB19 of B. burgdorferi in .lambda.FIX II. A 5.0 kb Bgl II fragment containing the complete fla gene of strain HB19 was subcloned into the plasmid vector pBR322 to yield recombinant plasmid pACA1. The nucleotide sequence of both strands of the flagellin gene and its 5' and 3' flanking sequences in pACA1 were determined by primer-directed sequencing of double-stranded pACA1 plasmid DNA (SEQ ID No: 1). The start of transcription of flagellin gene was identified by primer extension analysis of total RNA isolated from strain HB19 B. burgdorferi. The analysis revealed the following: (i) the coding region for the flagellin gene of strain HB19 from positions 58-1065; (ii) the transcriptional start site, the C at position +1, 57 bp distant from the start codon; (iii) the likely ribosomal binding site (RBS) as GGAGG at position 45 to 49; and (iv) the likely "-10" (GCTATT) and "-35" (CGTT) promoter boxes. The numbers in the top column refer to nucleotides (SEQ ID No: 1), those in the bottom column to amino acids (SEQ ID No: 2).

Detailed Description Paragraph Right (3):
In some circumstances, it may be desirable to mutagenize the starting population of B. burgdorferi, for example, with chemicals, such as nitrosoguanidine, or irradiation, such as gamma-rays, in order to increase the frequency of mutation. The mutagenized B. burgdorferi are then selected by cloning by limiting dilution or by colony formation as described above. Another procedure which could be used comprises transposon mutagenesis of the flagellin gene followed by selection in antibiotic-containing BSK medium without Yeastolate (BSK I), and further selection for flagella-less mutants as described above. With the aid of the present disclosure, one may also devise methods for preparing flagella-less strains by recombinant DNA technology, for example, in vitro mutagenesis of the cloned flagellin gene and transformation of the mutant gene back into the borrelia. The sequence of the cloned flagellin gene and the 3' and 5' flanking sequences is shown in FIG. 1 (SEQ ID No: 1). The cloned flagellin gene could be accompanied by an antibiotic selection marker to aid selection of transformants in broth medium or on solid medium. Antibiotic-resistant transformants would be examined as to flagella phenotype. With any of these procedures, the mutations may be in the gene itself or in the regulatory regions, such as the promoter or terminator, for the flagellin gene. More specifically, such mutations can include deletion of the entire coding region of the gene or portions thereof, deletion or mutagenesis of the ribosomal binding sequence (RBS), deletion or mutagenesis of the -10 and -35 promoter boxes, or insertion or deletion of DNA transcribed sequence of the gene such that a functional flagellar protein is not produced.

Detailed Description Paragraph Type 1 (5):
5. A. G. Barbour et al., A Borrelia-specific monoclonal antibody binds to a flagellar epitope, Infect. Immun. 1986; 52:549-54.

Detailed Description Paragraph Type 1 (48):
48. F. Sadallah et al., Production of specific monoclonal antibodies to Salmonella typhi flagellin and possible application to immunodiagnosis of typhoid fever, J. Inf. Dis. 1990; 161:59-64.

☐ 16. Document ID: US 5403718 A

L1: Entry 16 of 16 File: USPT Apr 4, 1995

DOCUMENT-IDENTIFIER: US 5403718 A
TITLE: Methods and antibodies for the immune capture and detection of Borrelia burgdorferi

Detailed Description Paragraph Right (6):
Escherichia coli, Shigella dysenterae, Shigella flexneri, Salmonella typhi, Salmonella typhimurium, Yersinia enterocolitica, Campylobacter jejuni, Helicobacter pylori, Clostridium perfringens, Clostridium botulinum, Staphylococcus aureus, Bacteroides fragilis, and the like.

Detailed Description Paragraph Right (19):
Antibodies useful in the immune capture method for isolating specific microorganisms are antibodies that react with external components of the microorganisms. Such external components include but are not limited to cell surfaces, surface coats, cell walls, slime layers, extracellular flagella, pili and the like. The antibodies are produced by immunizing mammals with the intact microorganism or with purified or partially purified external components of the microorganism. The antibodies are also produced by immunizing mammals with modified external components of the microorganism or using synthetic peptides, carbohydrates, lipopolysaccharides, glycoproteins, glycolipids or recombinant peptides or proteins, the structures of which one deduces from the naturally occurring microorganism. These antibodies are capable of binding and retaining intact microorganisms onto antibody-activated surfaces for isolation and subsequent cultivation. Preferred antibodies are antibodies that are species-specific and thus are capable of binding to a particular species of microorganism. The antibodies are polyclonal, or monoclonal, and may be a cocktail of antibodies with different reactivities for the microorganism of interest.

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((((flagell$ or flagellin or flagul$ or flagela) same (heterologous or foreign or epitope or paratope or mineotope or insertion or inserted or recombinant$)) or ((flagell$ or flagellin or flagul$ or flagela) near25(heterologous or foreign or epitope or paratope or mineotope or insertion or inserted or recombinant$))) and (typhi or typhus or para-typhi or para-typhus or paratyphi))	16

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